THE BIOLOGY, ECOLOGY AND CONSERVATION OF *Euphorbia clivicola* IN THE LIMPOPO PROVINCE, SOUTH AFRICA

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THE BIOLOGY, ECOLOGY AND CONSERVATION OF *Euphorbia clivicola* IN THE LIMPOPO PROVINCE, SOUTH AFRICA

BY

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ABSTRACT

The need to conduct a detailed biological and ecological study on *Euphorbia clivicola* was sparked by the drastic decline in the sizes of the Percy Fyfe Nature Reserve (Mokopane) and Radar Hill (Polokwane) populations, coupled with the discovery of two new populations; one in Dikgale and another in Makgeng village. The two newly (2012) discovered populations lacked scientific data necessary to develop an adaptive management plan. This study aimed to conduct a detailed biological and ecological assessment, in order to develop an informed management and monitoring plan for the four populations of *E. clivicola*.

This study entailed a demographic investigation of all populations and an inter-population genetic diversity comparison so as to establish the relationship between all populations of *E. clivicola*. The abiotic and biotic interactions of *E. clivicola* were examined to determine the intrinsic and extrinsic factors causing the decline in the Percy Fyfe Nature Reserve and Radar Hill population sizes.

Fire as one of the abiotic factors was observed to be beneficial to *E. clivicola* because it weakened their number one competitor, i.e., graminoids in populations with low or no grazing pressure. Fire also destroys refuge for rodents, which feed on the flowers of *E. clivicola,* as such, affecting reproduction success. An exponential growth was observed to be directly proportional to fire damage.

The populations of Percy Fyfe Nature Reserve, Radar Hill, and Dikgale were found to be genetically compatible. The genetic homogeneity makes intra-population restocking possible. Anthropogenic induced fragmentation proved to be detrimental to Radar Hill and Makgeng populations of *E. clivicola*, by reducing the Area of Occupancy of the species and hindering seed dispersal. Propagules of *E. clivicola* are dispersed down a gradient by surface water run-off, and therefore; slope angle and aspect directs the expansion of the *E. clivicola* populations. *Euphorbia clivicola* plants (71%) were observed to prefer north-facing gentle slopes of < 11°. Grass cover of > 60% negatively affects the reproduction success of *E. clivicola* by obstructing pollinators' access to flowers.

DNA data from this study indicate that the Percy Fyfe Nature Reserve population can be re-stocked with genetically attuned individuals from Radar Hill and Dikgale populations of *E. clivicola* at suitable habitats to prevent extinction. The exclusion of herbivores from the Radar Hill population triggered a negative domino effect (increased grass cover, refuge for herbivores, and amplified the density between individuals), as such alternative management actions need to be adopted for the population of Percy Fyfe Nature Reserve.

In order to halt the dramatic decline at Radar Hill population, an adaptive management plan that possesses three main focal points, which are; objectives, management, and monitoring is proposed. The population of Percy Fyfe Nature Reserve is so drastically reduced that it requires a more intensive approach; hence, a recovery plan is proposed. This plan takes into account the genetic similarity of three populations (Percy Fyfe Nature Reserve, Radar Hill and Dikgale), and model management and monitoring objectives to be more or less similar, so as to save time and resources. The three common components of monitoring the above mentioned populations are population size, canopy size, and density.

DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo for the degree of Master of Science has not previously been submitted by me for a degree at this or any other University, that it is my own work in design and in execution, and that all material contained herein has been duly acknowledged.

-- --

Seloba Ignitius Chuene Date

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DEDICATION

This work is dedicated to my late grandmother (Hedwig Raesetja Chuene), who taught me the art of hard work. "I know you wanted me to become a medical doctor, but I am one-step away from becoming a philosophical doctor" I hope you are proud of me "Ngoato 'mosadi".

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. GENERAL INTRODUCTION

1.1 South Africa's biodiversity

South Africa is a country with massive biodiversity, which comprises an astonishing variety of biomes, including: Savanna, Thicket, Grassland, Forest, Fynbos, Nama Karoo, Succulent Karoo and Desert (Rutherford and Westfall, 1994). Within these biomes there is enormous species diversity and endemism. This country contains between 250 000 and 1 000 000 species, many of which occur nowhere else. In the plant kingdom alone, at least 80% of the 18—20 000 species are endemic (Crane, 2006). The International Union for Conservation of Nature (IUCN Red List, 2014) recognises South Africa as the third most biologically diverse country in the world. South Africa is therefore regarded as the most floristically rich country in Africa. Thus, proper management and conservation strategies should be employed to preserve this prestigious heritage and lessen factors that lead to its extinction.

1.2 Extinction

According to Singh (2002), extinction of species is a natural phenomenon; however, during the second half of the 20^{th} century, species extinction rates reached an almost aberrant level in earth's history (Andreou *et al*., 2011), and the survival of many species is now threatened by unprecedented anthropogenic activity (Lienert, 2004). The most significant of which are processes that cause habitat degradation leading to habitat loss, over exploitation of natural resources, introduction of alien species and climate change (Barnosky *et al.,* 2012). Shah *et al.* (2008) indicated that there is a positive correlation between extinction rates of wild species and human activities. Not only loss of habitat, but also the subsequent fragmentation of (semi-)natural habitats seriously jeopardizes the survival of biodiversity intactness (Piessens and

Hermy, 2006). Singh (2002) estimated that the present mass extinction may be completed within as little as 200 years, and suggested that about 20% of all species are expected to be lost within 30 years, and 50% or more by the end of the 21st century. Adaptive management actions need to be integrated to halt the current extinction rates.

A key theme in environmental management is the problem of allocating an environment of a fixed size (such as land) for two competing and mutually exclusive uses; conservation and development (Leroux *et al*., 2009). It is therefore vital to prioritise research in these fields to provide policy-makers and practitioners with efficient tools to halt losses. Thus, an understanding of a threatened taxon's general life history characteristics, reproductive biology, demography and factors constraining population growth is accepted as a basis for the protection and restoration of species (Burne *et al*., 2003).

1.3 Threatened plant species

Threatened plant species are defined by their geographic range (large vs small), habitat specificity (wide vs narrow) and local population size (large vs small) (Rabinowitz, 1981). Rare plant species are defined as entities that occur in one or a few small populations, and are therefore confined to a single or only a few localities (Kruckeberg and Rabinowitz, 1985). *Euphorbia clivicola* R.A. Dyer fits both definitions.

2 RESEARCH PROBLEM

2.1 Demographic status of *Euphorbia clivicola*

Euphorbia clivicola is an endangered Limpopo Province endemic plant species. The species is restricted to four populations; one is protected within a Nature Reserve (Percy Fyfe Nature Reserve), while another occurs in a peri-urban area (Radar Hill) in Polokwane. Recently (2012), two additional populations were discovered, one around Kgwareng village in the Dikgale area and another one around Makgeng village near Boyne.

Demographic monitoring data collected for the protected population at Percy Fyfe Nature Reserve by the Transvaal Provincial Administration (Division of Nature Conservation) from 1987 to 1993, and again in 1996, indicated a drastic decline from 173 seedlings, 288 juveniles and 1460 adult plants in 1987 to zero seedlings, five juveniles and 160 adults in 1996 (Pfab and Witkowski, 2000). Pfab's (1997) data of this protected population indicated that 91% of the population had disappeared in less than 10 years, from an estimated 1921 individuals in 1987 to 165 individuals in 1996, with the probability of extinction calculated at 88% within the next 20 years starting from 1996 (Pfab and Witkowski, 2000). The urban population at Radar Hill has also declined, from an original estimate of 3000 individuals to 382 plants in 1996. These declines were attributed to loss of habitat and habitat fragmentation (Pfab, 1997).

2.2 Problem statement

Extinction of rare and endemic plant taxa is undoubtedly causing the deterioration of biological diversity worldwide. Rare species with only one or a few populations have a greater chance of going extinct because the probability of all populations disappearing is higher (Primack, 1993). The drastic decline in *E. clivicola* populations at Radar Hill (Polokwane) and Percy Fyfe Nature Reserve (Mokopane) has sparked concern amongst conservationists in Limpopo Province. Two newly discovered populations (Dikgale and Makgeng) lack scientific data necessary for conservation. Of the four populations of *E. clivicola*, only one is managed in a Nature Reserve (Percy Fyfe Nature Reserve), while the other three are unmanaged and unprotected. The management activities (Pfab, 1997) are inadequate, hence, the 91% decline at Percy Fyfe Nature Reserve population. The three unprotected populations (Radar Hill, Dikgale, and Makgeng) are threatened by development, which has lead to fragmentation of the population's habitats. A robust and informed management plan is required to conserve and improve or at least maintain all four populations of *E. clivicola in situ*.

3 RESEARCH QUESTIONS

a) What is the level of genetic diversity between populations of *Euphorbia clivicola*?
b) How do abiotic and biotic habitat features affect *Euphorbia clivicola* population biology and ecology?
c) What is the state of the new populations in terms of demographical, ecological and biological requirements?
d) What are the causes of the current drastic decline in Percy Fyfe Nature Reserve and Radar Hill populations?

4 LITERATURE REVIEW

4.1 The importance of conservation

The world is currently in the midst of a biodiversity crisis, with an estimated 2-25% of all species at risk of extinction due to anthropogenic causes (Singh, 2002). This has become a major problem in terms of saving our animal and plant diversity (Cullen *et al.*, 2001). Biodiversity, which incorporates all natural variability from the genetic level to landscape level, is of value to humans in that it provides essential goods, services, information, and is of aesthetic value (Wessels *et al*., 2003). Furthermore, the importance of future processes, such as altered dynamics of species interactions under global change scenarios, urban and infrastructure development, and habitat reduction and fragmentation, may increase in the future (Burgman, 2002). It is therefore very important to conserve biodiversity, whether it includes rare, endangered or common species, in order to guarantee the ongoing existence of species, habitats, biological communities, interactions between species and ecosystems, and ultimately mankind (Spellerberg and Sawyer, 1996).

Conservation of biodiversity incorporates aspects such as preservation, restoration and enhancement of the environment, and controlled utilization of our natural resources (Given, 1994). The three goals of conservation outlined by Pressey *et al*. (2003) are: a) The protection of biodiversity in priority areas, b) The promotion of sustainable use of biodiversity, and c) The strengthening of institutions, and the promotion of co-operative governance and community involvement in conservation. These should be incorporated in all management and conservation plans. When compiling biodiversity conservation and management plans, biological conservationists around the globe should take into account and adopt the mission from the Convention on Biological Diversity (2010), which is to take effective and urgent action to halt the loss of biodiversity in order to ensure that ecosystems are resilient and continue to provide essential services, thereby securing the planet's variety of life, and contributing to human well-being, and poverty eradication.

4.2 Small population paradigm

One of the most important challenges facing conservation biologists is the conflict of land use within areas that possess globally significant species diversity, because the same areas are generally also favoured by people (Fjeldsa and Rahbek, 1998). This challenge will ultimately result in numerous plant populations getting smaller (Ricketts *et al*., 2005).

4.2.1 Meta-population dynamics

The meta-population concept was introduced in ecological science by Levins in 1969 (Bulte and Van Kooten, 1999). It was defined as scattered populations of a species in a landscape, which are in contact via migration, and that are characterised by local population extinction and by colonisation of unoccupied sites (Hanski and Gilpin, 1997). A broader and more inclusive concept of population dynamics came into use, which includes both dispersal and spatial variation in habitat type and quality (Krohne, 2001). Meta-population is a collection of subpopulations interconnected by dispersal (Levins and Culver, 1971). This type of population structure applies to groups of subpopulations, which live in a landscape with habitats that vary in quality, and that occur in discrete patches (Schemske *et al.*, 1994).

Several meta-population dynamic models also predict a positive correlation between geographic range, which is measured as the number (or proportion) of patches occupied and population density within patches (Lawton, 1995). The dynamics and extinction risks of local populations are strongly influenced by abiotic conditions and biotic interactions, such as competition, herbivory and extreme climatic conditions (Milden *et al.*, 2006). Published literature has clearly illustrated the negative effects such extreme conditions have on populations. For example, fires and windstorms were two interacting factors that contributed to the demise of the Heath Hen, *Tympanuchus cupido cupido* Knowlton (Lindenmayer and Possingham, 1995).

Species may almost immediately respond to fragmentation or disturbances, but a time lag in response may also occur, creating an extinction debt; a condition in which, following environmental changes, populations and meta-populations are still present (Piqueray *et al.*, 2011). Hylander and Ehrlen (2013) argued that extinction debts can arise because: a) Individuals survive in resistant life-cycle stages long after habitat quality changes, b) Stochastic extinctions of small populations are not immediate, and c) Meta-populations survive long after habitat connectivity has decreased. Thus, it will be difficult to predict future effects on species richness or populations without baseline knowledge of the extent and speed of habitat loss or fragmentation (Cousins and Vanhoenacker, 2011).

Habitat patches differ in quality, size, and spatial arrangement. (Re)colonization and migration depend on the distance between habitat patches, and for the maintenance of meta-population dynamics as a whole (Giles and Goudet, 1997). Krohne (2001) is of the opinion that the dispersal rate of a subpopulation will be determined by the species' vagility and the distance separating the subpopulations. In meta-populations, systems of subpopulations in suitable, discrete habitat patches interact via dispersal of individuals moving in the matrix. Such systems are buffered against extinction by gene flow between subpopulations, rescue effects or recolonisation after local extinction events (Baguette, 2004).

The abundance and persistence of many species may be largely controlled by a single limiting resource, which is often related to the amount of suitable habitat that is available (Gilpin and Hanski, 1991). Gilpin and Hanski (1991) further noted that individual members of a population, or patches of a meta-population, affect each other only indirectly, through the consumption of the limiting resource. In addition, two different species with similar growth forms in a community can also compete with each other for the one limiting resource.

4.2.2 Genetics

Habitat destruction and consequently habitat loss, leading to small and isolated populations with their own genetic makeup, is considered to be among the most important causes of plant species decline and extinction (Heinken and Weber, 2013). Genetic diversity within and between small populations of endangered species, are critically important for any conservation program. The long-term survival of such a species is strictly dependent on the maintenance of sufficient genetic diversity to facilitate adaptations to long-term environmental stochasticity (Chen *et al.*, 2009), their ability to disperse into new habitats, and to evolve *in situ* based on their genetic variance (Zhu *et al.*, 2009). Genetic homogeneity of such populations is thought to reduce the ability of populations to adapt to environmental changes for survival (Badfar-Chaleshtori *et al.*, 2012). Isolated small population patches are often extinction prone, because of inbreeding in combination with demographic and environmental stochasticity (Eriksson *et al.*, 2013).

Estimation of genetic variation and structure using molecular markers has become a common approach in protection of endangered species (Zhang and Zhou, 2013). Understanding genetic variation within and among populations is essential for the establishment of effective conservation practices such as relocation for rare and endangered species (Liu *et al*., 2011). Translocation of genetically viable species along various populations can be a valuable ecological mitigation or conservation tool and also promotes gene flow (Griffith *et al*., 1989).

4.3 Declining population paradigm

The management of threatened species is an important way in which conservationists can intervene in the extinction process and reduce the loss of biodiversity (Norris, 2004). When designing a management strategy that will help recover an endangered species, it is essential to identify agents responsible for the decline of a population (Peery *et al.,* 2004). Generalization is not a characteristic of this paradigm, and it is therefore characterized by a lack of theory (Pfab, 1997).

According to Norris (2004), the declining-population paradigm encompasses two main areas of theory: the causes of extinction, and the means by which the causes of decline are diagnosed. Our understanding of variation in extinction rates between taxa and the ecological factors that influence this variation is still relatively poor. Integration of the two paradigms (small population and declining population) has proven to be difficult, because the small population paradigm mainly deals with the proximate causes for populations going extinct even when protected, while the declining population paradigm considers ultimate reasons for some species becoming rarer than others at the outset (Saether *et al.,* 1996).

Human activities have changed between one-third and one-half of the earth's land surfaces, and are leading to substantial and growing modification of the earth's biological resources (Lienert, 2004). Habitat fragmentation, leading to small and isolated populations, is considered to be among the most important causes of plant species extinctions (Heinken and Weber, 2013). Thus, it is not surprising that destruction and degradation of habitats are often cited as the leading threats to biodiversity (Lawlor *et al.,* 2004). Habitats could be degraded by agents, such as a change in the fire regime (Pfab and Witkowski, 1999a), grazing by livestock (Bradshaw, 1981), and felling a patch of forest or draining a wetland (Caughley, 1994).

Wilcove *et al*. (1998) have outlined the two major categories of threats to biodiversity as: habitat destruction, and the spread of alien species. Natural causes of native species extinction are mostly triggered by habitat alterations and human induced stresses. Genetic models are based on the premise that inbreeding accumulation will reduce fitness and accelerated extinction of small and isolated populations will arise (Mills and Smouse, 1994). When fitness of a particular species is compromised; demographic, environmental stochasticity and other factors start to take their toll. A single feature of the environment cannot often be responsible for annihilating an entire population. For example, photoinhibition is dramatically increased when high light exposure is accompanied by water stress, or unusually low or high temperatures (Chapin III *et al.,* 1987).

Alien species introduced either intentionally or unintentionally by people, eliminate native species by competing for limiting resources, or destroying their habitat (Marom, 2006). Invasive species have tremendous consequences for native biodiversity due to the direct effects of interspecific competition, disease, and herbivory, or indirect effects of hybridisation and introgression between alien and native species (Bleeker *et al*., 2007).

4.3.1 Population biology and the effects of abiotic-biotic features

The ultimate goal of conservation biology is to maintain the evolutionary potential of species by maintaining natural levels of diversity, including genetic, species and ecosystem diversity (Barrett and Kohn, 1991). Population biology is the study of a group of living organisms present at the same place and time (Feldhamer *et al.,* 1999), and it is concerned with spatio-temporal variation in numbers and sizes of individuals (Harper, 1977).

Interactions between abiotic and biotic features can have various effects on the biology and ecology of the populations.

a) Abiotic features

Africa is often referred to as a 'fire continent' because of widespread anthropogenic fires that annually burn the savanna vegetation (Oba *et al*., 2005). Traits, such as resprouting, serotiny and germination by heat and smoke, are adaptive in fire-prone environments (Keeley *et al.,* 2011). Keeley *et al*. (2011) further alluded to the fact that plants are not adapted to fire *per se* but rather to fire regimes. That being said, inconsistency of fire frequency may threaten species. Fire is beneficial in nutrient deficient soils, as it accelerates nutrient recycling.

Soil is a primary substrate for plant development. Above-ground and below-ground components of terrestrial ecosystems are closely related (Rodriguez-

Loinaz *et al*., 2008). Most plants require a similar balance of resources: energy, water, as well as macro- and micro-nutrients to maintain optimal growth (Chapin III *et al*., 1987).

b) Biotic features

Herbivore optimizations are positive effects of grazing on plant productivity and fitness. Plant traits that evolved due to mutualism involving herbivores are: high palatability, basal meristems, increased longevity, increased shoot production, vegetative growth and reproduction (Belsky, 1986). The negative effects of herbivory are evident when the predation of reproductive or vegetative parts decreases or reduce both plant fecundity and survival, which ultimately lead to reduced plant fitness. Many plant species have developed complex chemical and physical defences against herbivores (Andrieu *et al*., 2011). These defences are mostly visible on the subsequent offsprings of the individuals that experience the stress.

Habitat fragmentation often disrupts mutualistic plant-animal interactions such as those between plants and their pollinators, which are crucial to reproduction (Kolb, 2008). Reproduction is the process that translates the current genotypic array into that of subsequent generations (Loveless and Hamrick, 1984). That being said, disrupting plant-pollinator relationship may lead to the demise of future generations.

Both phenomenological and mechanistic models of competition demonstrate that competing species will coexist when intraspecific competition exceeds interspecific competition, a situation that has generally been presumed to result from abiotic nich differentiation (Bever *et al.*, 1997).

Competition may rise due to a limiting factor such as; habitat, macro- and micro-nutrients, energy, or pollinators. Intraspecific competition is the competitiveness of members of the same species for the same resource, while interspecific competition is the competitiveness of individuals of different species over the same resource (García-Cervigón *et al*., 2013).

4.3.2 Population ecology

Conservation ecology is a new paradigm of ecology that not only scientifically contributes to international and intranational social movements aimed at maintaining earth's biodiversity, but is also committed to adaptive ecosystem management, which is indispensable to the intergenerational long-term sustainability of mankind (Washitani, 2001). Population ecology plays a central role in conservation ecology, and examines interactions between organisms and their environment. Population ecology studies aim to address the following questions (Krohne, 2001):

a) What are the characteristics of a population?

   The relationships between plant population size, fitness and genetic diversity are of fundamental importance in plant ecology, evolution and conservation (Leimu *et al*., 2006).

b) Which population parameters can be measured?

c) How do populations differ in aspects such as density and age distribution?

d) How do populations expand?

e) What are the patterns of population increase or decrease?

f) How are population numbers controlled?

g) What factors determine the limits of population size?

The data that is usually collected in an ecological investigation of rare and threatened plant species include various biotic and abiotic factors (Krohne, 2001). Biotic factors are aspects which are related to life or living factors like plants, animals, fungi, protest and bacteria (Given, 1994).

Pfab (1997) noted that the type of data that is frequently collected during ecological studies of rare and threatened plant species includes:

i. A description of the abiotic environment, including climatic data, geology, geomorphology, soil types, soil analyses, topographic position, slope and aspect, and fire regime and other disturbances.

ii. A description of the biotic environment, including over-storey and under-storey shading and litter thickness, vegetation physiognomy and species composition, grazing and browsing impacts, disease, alien plant infestations, and human impacts.

An example of the effects of abiotic factors on plant population was illustrated by Dunson and Travis (1991), on the competitive relations among *Galium* (bedstraws) and the effect of different soil types. In the field, *Galium hercynicum* Weigel was found on acidic soils and *Galium pumilum* Murray on calcareous soils. When grown together, the acidophilic species dominates on acidic soil and the calcareous species on basic soil. This experiment demonstrated the reversal of asymmetric competition by changes of an abiotic variable (soil chemistry), and the role of those effects in determining species distributions.

5 EUPHORBIACEAE

5.1 Historical background

The botanical name *Euphorbia* derives from Euphorbus, the Greek physician of King Juba II of Numidia (52—50 BC—23 AD) (White *et al.*, 1941). He is reported to have used a certain plant, possibly Resin Spurge *Euphorbia resinifera*, as a herbal remedy when the king suffered from a swollen belly. Carolus Linnaeus assigned the name *Euphorbia* to the genus in honour of the physician (Wales, 2001).

5.2 *Euphorbia*

The genus *Euphorbia* represents a diverse group of plant species belonging to the Spurge family (Euphorbiaceae) (Wales, 2001). *Euphorbia* is distributed worldwide, and varies in height from dwarf succulents to trees as tall as 20 m. These spurges achieve their greatest diversity in the arid areas of Africa and Madagascar where many of them are cactus-like succulents; most of these are rare and endangered due to human encroachment (Berry *et al*., 2006). The genus is comprised of approximately 2 160 species, which makes it one of the largest genera in the plant kingdom (White *et al.*, 1941).

5.3 World Conservation Union classification

The International Union for the Conservation of Nature's (IUCN) Red Data List of Threatened Species lists 171 species of the genus *Euphorbia* as species of conservation concern (IUCN, 2007). Of these 171 species, 51 (29%) are listed in the upper conservation categories of Endangered or Critically Endangered (IUCN, 2007). Africa has the dubious distinction of having 72 (42%) of the 171 threatened *Euphorbias* worldwide, of which 25 (15%) species are listed as Endangered or Critically Endangered (IUCN, 2007). One of the major reasons for the endangered status of so many *Euphorbias* is habitat destruction as a result of the ever increasing human population. Associated with habitat destruction is a range of factors related to human activities, e.g. collector pressure, trampling by livestock and humans, destruction by vehicles (recreational and commercial), development (urbanisation) and pollution (Pfab and Witkowski, 2000).

5.4 South African distribution

It is estimated that about 10% of the world's *Euphorbia* species can be found in South Africa (Fourie, 1983; Wales, 2001). *Euphorbia* species can be found in all the major biomes in South Africa, and can reach extreme densities in places like the Eastern Cape's Noorsveld, e.g. *Euphorbia coerulescens* Hort. and along the western coast of South Africa as far north as the Richtersveld region (Van Jaarsveld *et al.*, 2006). The accelerated rate of extinction of species discussed earlier also threatens several members of the Euphorbiaceae in South Africa. Many members of this family have extremely small ranges and narrow habitat requirements, and are threatened as a result of various factors discussed earlier. These include, amongst others, *Euphorbia barnardii* A.C. White, R.A. Dyer and B. Sloane, *E clivicola* R.A. Dyer, and *E perangusta* R.A. Dyer (Hilton-Taylor, 1996).

Only a few South African *Euphorbia* species have been studied in sufficient detail to develop a conservation management plan based on sound scientific principles. Pfab and Witkowski (1999a, 1999b & 2000) studied the critically endangered *Euphorbia clivicola*, whereas Knowles and Witkowski (2000) studied the population biology and ecology of the endangered *Euphorbia barnardii,* and made recommendations for its effective management. Several other Red Data listed *Euphorbia* species are almost entirely unknown, and are only known from distribution records or historic monitoring records of the Transvaal Provincial Administration, e.g. *Euphorbia grandialata* R.A. Dyer, *E. groenewaldii* R.A. Dyer*, E. louwii* L.C. Leach*, E. perangusta* R.A. Dyer*, E restricta* R.A. Dyer*, E. rowlandii* R.A. Dyer*, E. tortirama* R.A. Dyer and *E. waterbergensis* R.A. Dyer (White *et al.*, 1941; Act No. 7 of 2003).

From the above information it is evident that several members of the genus are in dire need of conservation efforts. However, our attempts at conserving these species are hampered by a lack of knowledge about their general biology and ecological requirements (Fourie, 1983; Witkowski and Liston, 1997). Moreover, their potential for providing novel compounds for medicinal purposes is virtually unknown (Pfab, 1997).

5.5 *Euphorbia clivicola*

5.5.1 Morphological description

Bruce *et al.* (1951) described *E. clivicola* as a dwarf spiny perennial succulent (Figure 1.1), with the main stem and roots merging into one another and forming an underground tuberous body about 15 cm long and 2—3 cm thick, tapering to the base, and with a few slender secondary roots. Stems are repeatedly-branched and retracting further underground as the plant enlarges, only the young terminal branches remain above ground. Branches are yellowish-green, congested into a dense mass of about 2—6 cm. If the plant is shaded by grass of 15 cm or more, the branches become thick towards the base, and narrower towards the apex. The branches are four-angled, and have more or less decussate arrangement of the tubercles and paired spines. Inflorescence; cymes are solitary, emerging from the apical flowering eyes of the branches, sessile, usually consisting of three cyathia, the central one being male, with two bisexual cyathia laterally disposed on very short peduncles or sessile. The fruit contain three lobes with a single seed in each lobe.

Figure 1.1 Flowering and fruiting *Euphorbia clivicola* from the Dikgale population.

5.5.2 Conservation status

Euphorbia clivicola is restricted to only four small populations of which two are known and have been superficially studied, while the other two populations were recently (2012) discovered in Limpopo Province, South Africa; thus, it should be regarded as a rare species because of its small geographical range. *Euphorbia clivicola* has specific habitat requirements, and there is a good possibility of extinction if no intervention is initiated. Conservation and management of threatened species requires a thorough understanding of their spatial distribution and demography which should form the basis of management and monitoring programmes conducted at regular intervals (Schemske *et al*., 1994). Our knowledge of *E. clivicola* is limited to a study by Pfab (1997), based on two of the four populations (Percy Fyfe Nature Reserve and Radar Hill).

6 RATIONALE OF THE STUDY

Rare and endemic plant taxa are much more susceptible to extinction than other taxa because of the many unique attributes that they have (Primack, 1993). For example, they may:

a) Cover a small range.

b) Comprise of one or a few populations.

c) Possess low population densities.

d) Have specific habitat requirements.

Isolated populations with a small number of individuals tend to lose genetic variability, which in turn increases their risk of extinction (Marrero-Gomez *et al*., 2003). The loss of genetic variation may result from genetic drift and/or inbreeding, and it must be highlighted that inbreeding does not lead to a loss of alleles, but rather redistributes alleles from heterozygous to homozygous combinations (Oostermeijer *et al*., 2003). Small and fragmented populations are sometimes threatened by stochastic events (Huenneke, 1991).

The above mentioned attributes also apply to *E. clivicola* because of its narrow global distribution range, small number of populations, small population sizes, present and future threats facing the long-term survival of the species, and collector pressure (Pfab and Witkowski, 2000). Because of these attributes the species is rendered susceptible to anthropogenic and stochastic events (Wales, 2001).

Two new populations; one in the Dikgale area, Kgwareng village, and the other at Makgeng village near Boyne in Limpopo Province were recently (2012) discovered; thus, baseline data is urgently needed to construct an adaptive management plan for the rare and endangered *E. clivicola*.

More than a quarter of South Africa's *Euphorbias* are listed in the Red Data List of Southern African Plants (Raimondo, 2011), but not all of them are listed in the IUCN Red List of Threatened Species, mainly because most of these species are poorly known. Furthermore very little information is available on their biology, ecological requirements (Pfab, 1997), and environmental factors influencing their spatial and temporal distribution (Witkowski and Liston, 1997). Witkowski *et al.* (1997) advocated that more research and conservation efforts should be directed towards these areas.

According to Washintani (2001), maintaining diversity and complexity of the ecosystem is an intermediate goal for sustainability. Planning and monitoring are hard to implement without more concrete targets, such as preventing the extinction of populations or species. Therefore, at a local scale, studies of threatened species associated with locally unique habitats are important.

7 GENERAL AIM AND OBJECTIVES

7.1 Aim

To determine inter-population genetic relationships, investigate abiotic and biotic factors that affect the persistence of *Euphorbia clivicola in situ,* and to formulate an adaptive management plan for the conservation of this threatened plant species.

7.2 Objectives

The objectives of this study were to:

a) Investigate the genetic relationship amongst all four populations of *Euphorbia clivicola*. Findings will establish if this is a single population that was fragmented by development, or if the populations are genetically different.

b) Determine the abiotic factors affecting the existence of *Euphorbia clivicola*. This information will assist in formulating an informed management plan.

   i. Assess the demography of all populations of *Euphorbia clivicola.* This will give a clear indication of the state of the health, demography and recruitment capacity of all known populations of *Euphorbia clivicola*.

   ii. Measure the effects of fire on the vegetative responses and recruiting ability of *Euphorbia clivicola*. Data collected will aid in validating Pfab and Witkowski's (1999a) recommendations of a fire regime for this species.

   iii. Investigate the abiotic cover features (bare ground, stone and fixed rock) associated with *Euphorbia clivicola*. The information gathered will give insight on the habitat requirements for *Euphorbia clivicola* introduction.

   iv. Determine the aspect and slope that *Euphorbia clivicola* inhabits. This will aid in understanding the habitat requirements of *Euphorbia clivicola*.

   v. Characterise the physical and chemical aspects of the soil that *Euphorbia clivicola* grows on. This will determine the abiotic stresses influencing the populations of *Euphorbia clivicola*.

c) Investigate the biotic factors affecting the existence of *Euphorbia clivicola*. The information gathered will aid in formulating a robust management plan.

   i. Measure the biotic cover features (grass, forbs, shrubs, and dead vegetation) associated with *Euphorbia clivicola*. The data will assist in the identification of any interspecific competitors.

   ii. Determine the effects of herbivory on *Euphorbia clivicola*. Data will aid in validating Pfab's (1997) recommendation of excluding herbivores at the Percy Fyfe Nature Reserve *Euphorbia clivicola* population.

d) Construct a management plan for all four populations of *Euphorbia clivicola* in an effort to mitigate the current drastic decline in the population sizes.

8 SCOPE AND LIMITATIONS OF THE STUDY

This study aimed to generate baseline data for the two newly discovered populations of *E. clivicola* located in Dikgale and Makgeng villages, in Limpopo Province, South Africa. The study also aimed to determine the extrinsic and intrinsic factors contributing to the drastic population decline at the two formally known populations (Percy Fyfe Nature Reserve and Radar Hill) reported by Pfab (1997). Data collected for rare species over a long time will lead to comprehensive conservation and monitoring plans. Lack of demographic data from the two formally known populations (Percy Fyfe Nature Reserve and Radar Hill) was a limiting factor in our study. This was due to the absence of a monitoring plan and poor management of the species by conservation agencies in Limpopo Province.

The population in Dikgale and the upper population of Makgeng were too large to conduct a total species count; hence, the Point Centre-Quarter method was used to estimate the population density and the demography.

9 DISSERTATION FORMAT AND LAYOUT

Chapter 1: Introduction and Literature Review

This chapter is a general introduction on conservation of rare plant species and biodiversity (*sensu lato*) in general. Components or factors necessary to assemble a comprehensive conservation and management plan for such species were broken down. Relevant data for such research projects is outlined. Challenges faced during this project and the extent of work is outlined in the scope and limitations. Rationale for selecting this research project is also included in this chapter, where reasons and motivation behind the project are stated.

Chapter 2: Study sites

This chapter describes and discusses the four study areas in terms of their altitude, geology, climate, management activities, flora and history. In addition, this study also highlights the rationale behind the establishment of the Percy Fyfe Nature Reserve.

Chapter 3: DNA analysis

Potential genetic risks associated with rare and/or endangered plants and small populations are discussed using theory and available data performed using the Inter-Simple Sequence Repeats Technique. Genetic diversity among all four populations of *E. clivicola* is presented.

Chapter 4: The effects of abiotic factors on the population biology and ecology

Direct and indirect effects of abiotic factors on the vigour, demography, density, regeneration capacity of all four populations of *E. clivicola* are documented in this chapter.

Chapter 5: The effects of biotic factors on the population biology and ecology

Results and discussion of the interspecific competition study between *E. clivicola* and graminoids are presented. The effects of herbivory on *E. clivicola* are also outlined.

Chapter 6: Conclusion and recommendations

Recommendations that will contribute to the proper conservation and recovery of the species are presented. An adaptive management plan was designed, using the data obtained from Chapters 3—5. The plan was constructed in line with the vision(s) and mission(s) of the Department of Limpopo Economic Development, Environment and Tourism (LEDET).

CHAPTER 2

STUDY SITES

1 STUDY AREAS

Euphorbia clivicola has been documented (Pfab, 1997) to occur in two localities within the Limpopo Province, namely; Percy Fyfe Nature Reserve, near Mokopane and Radar Hill in Polokwane (Figure 2.1). Percy Fyfe population also serves as the type locality for the species (Fourie, 1989). Radar Hill is an un-conserved area, while Percy Fyfe is a conservation area. Two additional, but hitherto undocumented, localities have recently (2012) been found. One locality is at Kgwareng village in the Dikgale area, and the other one is near Boyne at Makgeng village. None of the latter two localities fall within a conserved area (Figure 2.1).

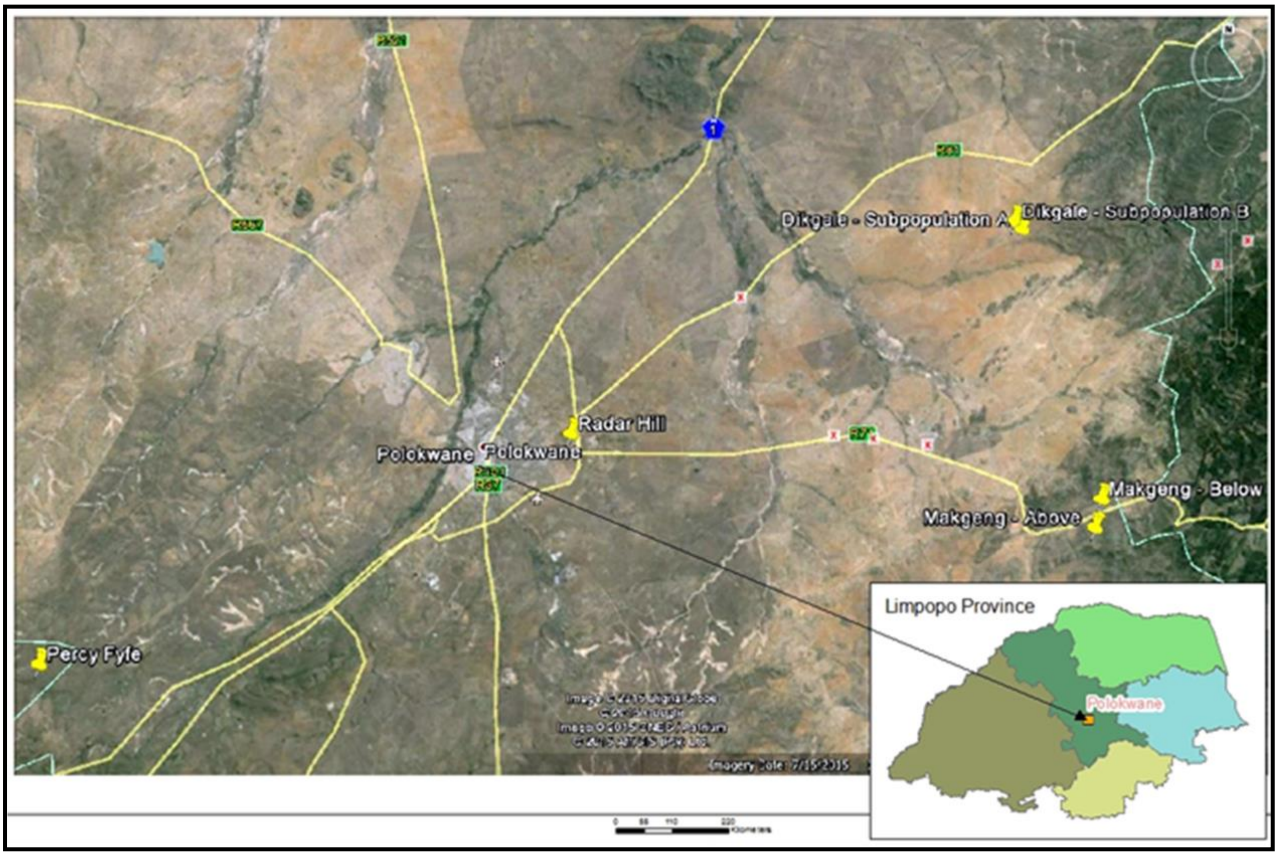


Figure 2.1 Localities of the four *Euphorbia clivicola* populations (Google Earth^{TM}, 2014).

1.1 Percy Fyfe Nature Reserve

1.1.1 Location

The Percy Fyfe Nature Reserve (PFNR), one of the oldest nature reserves in the province (Percy Fyfe Nature Reserve Strategic Plan, 2013), is located in the Waterberg District of Limpopo Province, South Africa. It is situated approximately 45 km south-east of Polokwane, 30 km to the north-east of Mokopane and 250 km north of Pretoria. The PFNR is located approximately 18 km to the east of the Witvinger Nature Reserve. The reserve covers an area of 2 918 ha, and is located over portions of the following farms: Rotterdam No. 12 KS, Lunsklip No. 7 KS and Dr iefontein No. 9 KS (Figure 2.2).

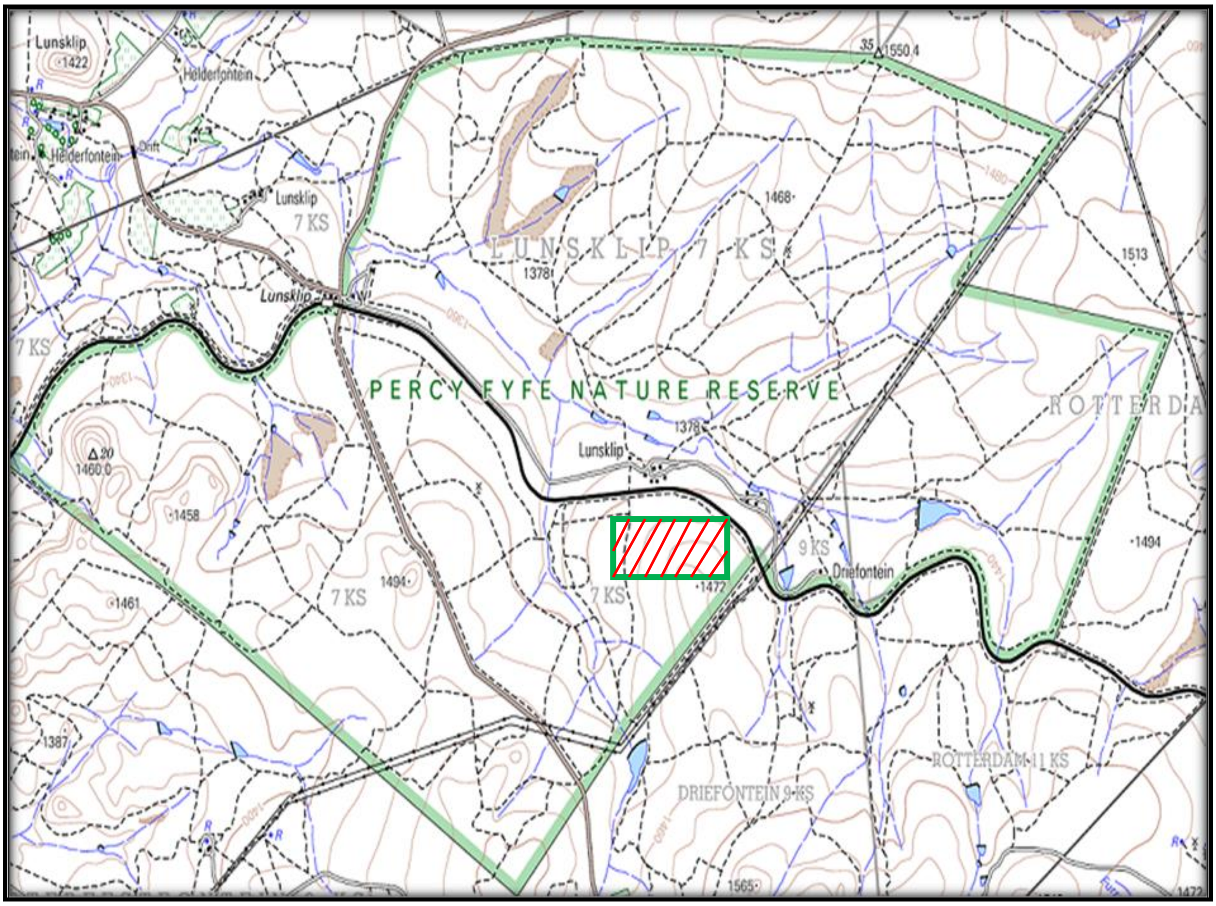


Figure 2.2 A 1:50 000 topographic map of Percy Fyfe Nature Reserve. Chevroned area indicates the *Euphorbia clivicola* population.

The north-facing slope of the hill at Percy Fyfe Nature Reserve which is dominated by graminoids and also where *Euphorbia clivicola* locality (Figure 2.3).

Figure 2.3 Location of *Euphorbia clivicola* population at Percy Fyfe Nature Reserve.

1.1.2 History background

One of the oldest nature reserves in the Limpopo Province, Percy Fyfe Nature Reserve was officially proclaimed as a game reserve and native flora reserve in 1954. The following information provides a brief summary of the history of the reserve, taken from Percy Fyfe Nature Reserve Strategic Plan, 2013.

The farm Lunsklip No. 7 KS was purchased by Mr Percy Poynton Fyfe in 1912. After purchasing the property, he immediately implemented a soil conservation programme. This programme consisted of dividing the property into 13 different camps, and conserving the indigenous plant and animal species associated with each camp, as well as preventing veld fires.

The property owner commenced with sheep farming on this property in the 1930's - a venture that was unsuccessful as a result of the high rate of predation of lambs by Black-backed Jackal (*Canis mesomelas*). Later the operation was switched from sheep farming to conservation and game farming, and Blesbuck (*Damaliscus pygargus phillipsi*) was introduced to the area, leading to the establishment of one of the largest and most well-known herds of Blesbuck in the former Transvaal Province.

The Fyfe couple did not have any children of their own, so they invited children from the local community to the farm during the school holidays in order to provide them with some basic environmental education. With no heirs to inherit the farm, Mr Fyfe became concerned about the future of the farm and decided to donate the property to the former Transvaal Nature Conservation Division (TNCD) in 1953. The title deed formalising this donation stipulated that the Fyfe couple could continue harvesting the fruit orchards on the farm, whilst the TNCD took over all management aspects with regard to the natural fauna and flora of the property. Another condition stipulated in the title deed stated that young people should always be given the opportunity to visit and learn from the PFNR, in order to increase conservation awareness.

Once the property was under the management of the TNCD, a game-proof fence was immediately erected and game populations, including Tsessebe (*Damaliscus lunatus*), Cape Buffalo (*Syncerus caffer*), Sable Antelope (*Hippotragus niger*) and Roan Antelope (*Hippotragus equines*), were introduced for breeding purposes.

The Blesbuck population established on the property by Mr Fyfe was removed by the TNCD in 1954, since the reserve does not fall within the natural distribution range of this species. The Sable Antelope population introduced to the reserve by the TNCD was also removed in 1994 as a result of the losses experienced from predation.

1.1.3 Climate

The PFNR receives summer rainfall and experiences cold, dry winter months (Percy Fyfe Nature Reserve Strategic Plan, 2013). Data received from the South African Weather Service (Mokopane weather station), indicate that the minimum daily temperature can drop as low as 5.6ºC in winter, and reach a mean maximum of 31ºC during summer (Figure 2.4).

According to Pfab (1997), the reserve received an average annual rainfall of 595 mm per year from 1970 to 1995, the bulk of which is received during the summer months. The fifteen year rainfall summary (Figure 2.5) reveals that the years 2002 and 2003 received the least amounts of rainfall of 132 mm and 138 mm, respectively. The year 2010 received 543 mm rainfall, which is the greatest in the 15 year summary period (1998-2012). Rainfall data from Mokopane weather station was absent in some of the months of 1999, 2001, and 2006, hence the years were excluded in the data presentation (Figure 2.6).

Mokopane experiences minimum mean wind speed of 1.01 m/s in July and a maximum of 3.84 m/s in October (Figure 2.7). Humidity recorded from the Mokopane weather station showed a monthly mean of 66% and a daily average that ranges from 54% in September to 72% in March (Figure 2.8).

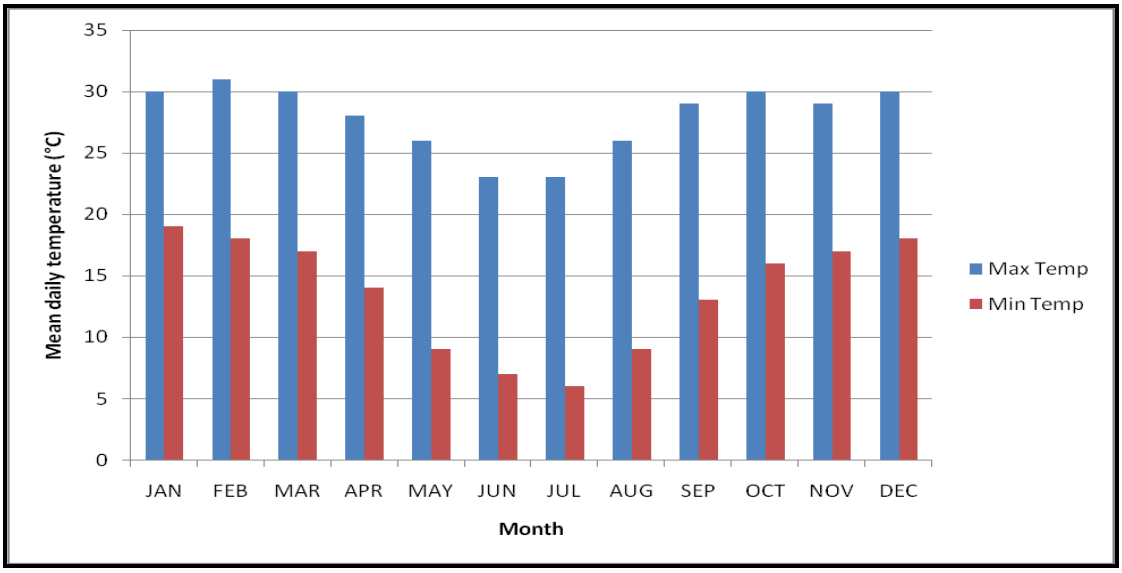


Figure 2.4 Mean minimum and maximum daily temperatures for Mokopane (1998 to 2012). Data was obtained from the South African Weather Service.

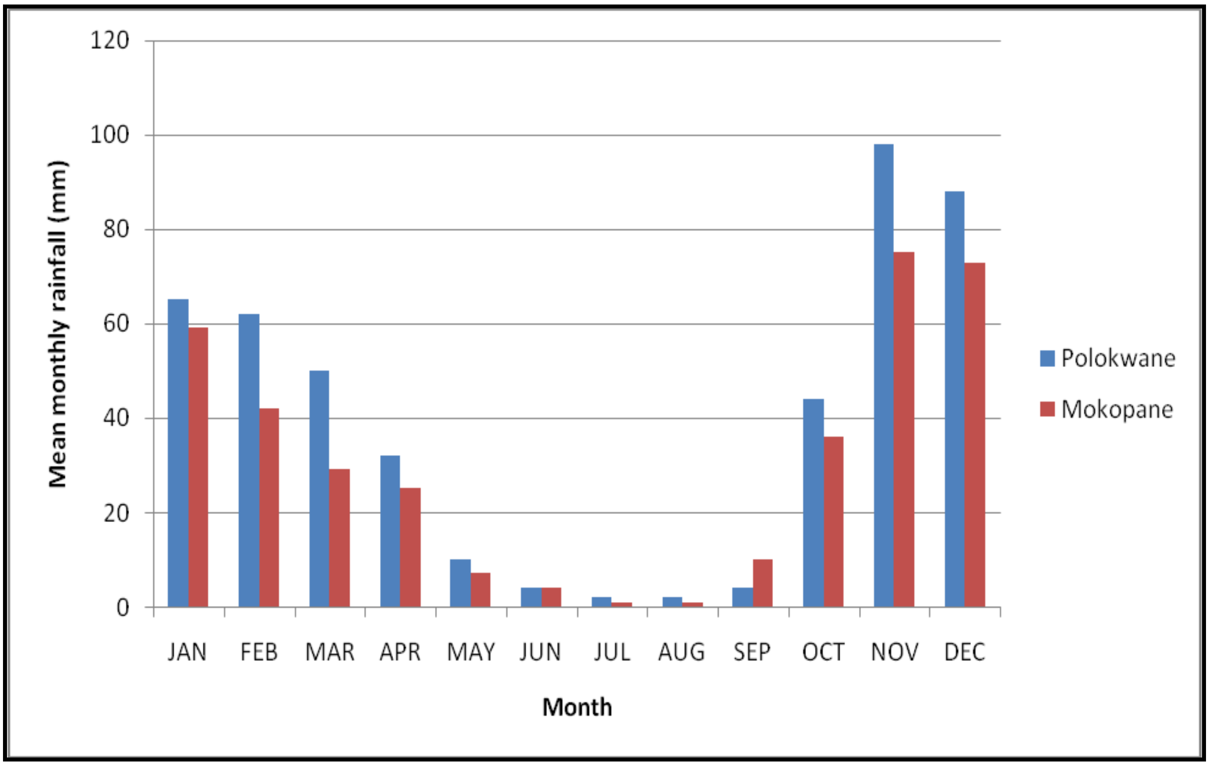


Figure 2.5 Polokwane and Mokopane mean monthly rainfall (1998 to 2012). Data obtained from the South African Weather Service.

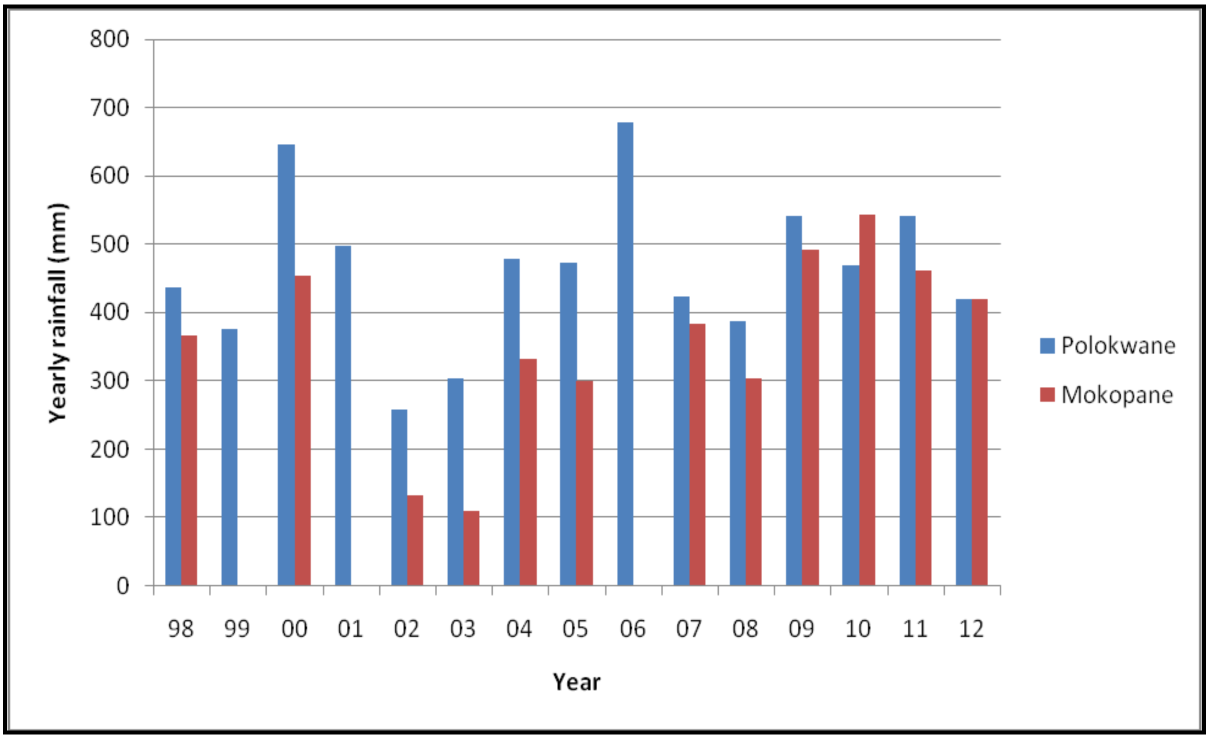


Figure 2.6 A (1998 to 2012) rainfall summary for Polokwane and Mokopane.

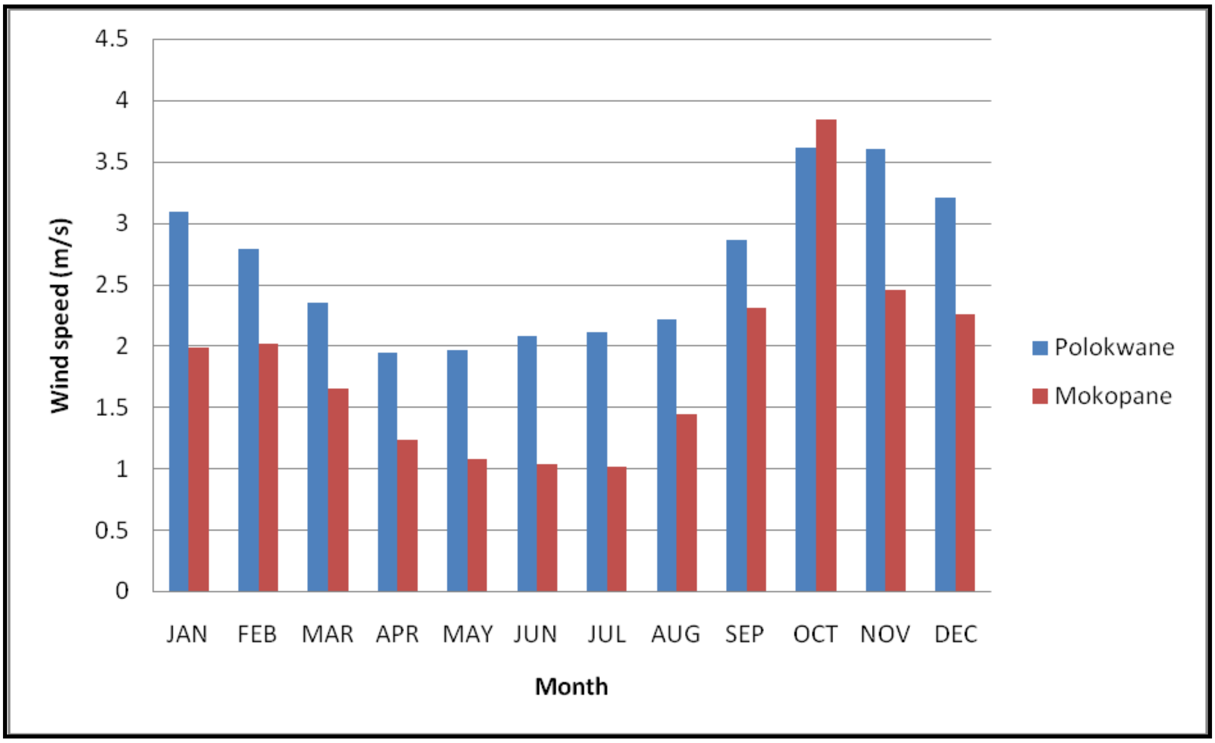


Figure 2.7 Mean daily wind speed for Polokwane and Mokopane.

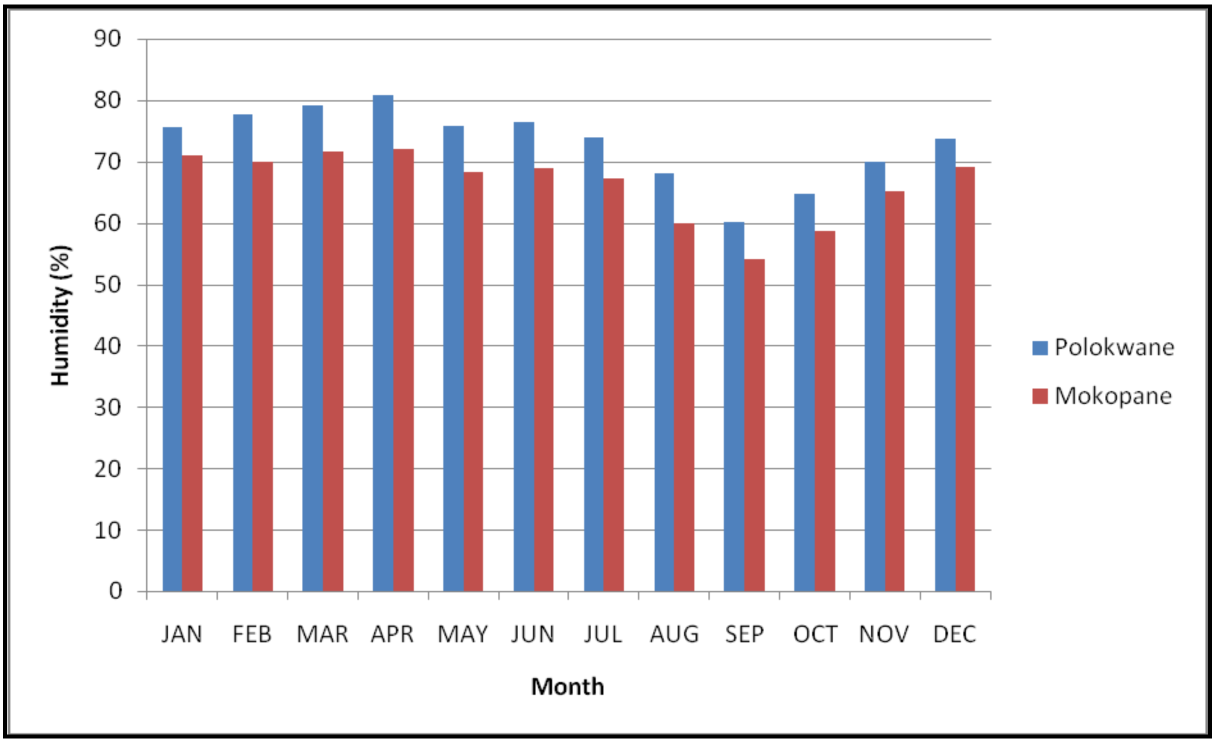


Figure 2.8 Average daily relative humidity for Polokwane and Mokopane.

1.1.4 Geology

Pfab (1997) described the geology as Archaean Granite. The Percy Fyfe Nature Reserve Strategic Plan (2013) noted that the reserve's geology is dominated by migmatites and gneisses of the Hout River Gneiss and the Turfloop Granite.

1.1.5 Vegetation

The area consists of undulating plains covered with a short open tree layer and a well-developed grass layer. Scattered trees occasionally occur at higher altitudes. The vegetation of this area falls within the Polokwane Plateau Bushveld (Mucina and Rutherford, 2006).

Notable tree and shrub species that occur in the area include; *Acacia caffra, A. permixta* and *A. rehmanniana* (trees); *Aloe marlothii* subsp. *marlothii* (succulent); *Acacia hebeclada* subsp. *hebeclada, Gymnosporia senegalensis* and *Combretum hereroense* (shrubs); *Anthospermum rigidum* subsp. *rigidum, Gymnospermum glaucophylla* and *Hirpicium bechaunense* (low shrubs). The herbaceous layer is comprised of *Aristida diffusa, Brachiara nigropedata, Digitaria eriantha* subsp. e*riantha* and *Eragrostis curvula* (grasses); *Felicia mossamedensis, Hermbstaedtia odorata* and *Pollichia campestris* (herbs) (Percy Fyfe Nature Reserve Strategic Plan, 2013).

1.2 Radar Hill - Polokwane

1.2.1 Location

The site is located on the southwest end of the suburb Sterpark and the northwest end of suburb Faunapark in Polokwane, at an altitude ranging from 1100 to 1500 m above sea level (Figure 2.9).

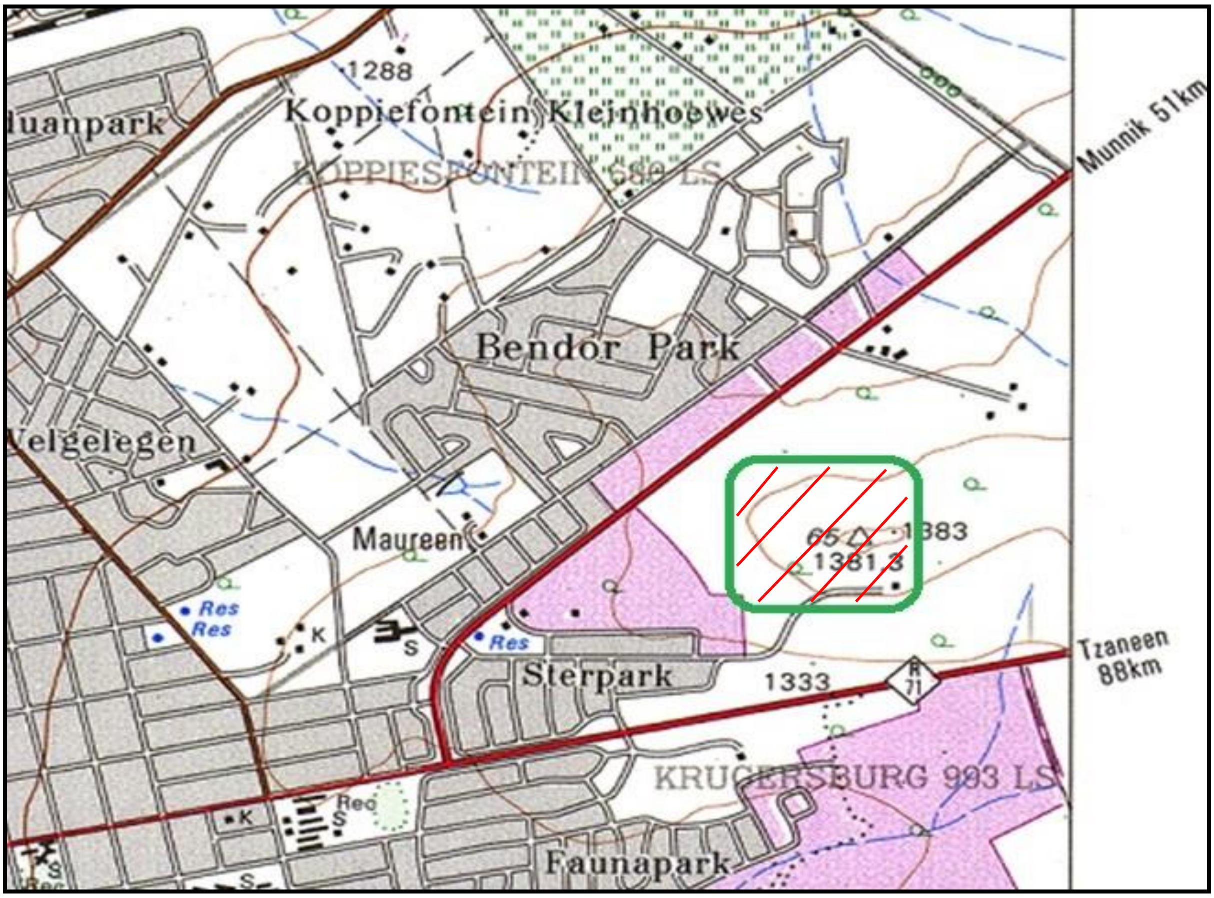


Figure 2.9 A 1:50 000 topographic map of Radar Hill location. Chevroned area indicates the *Euphorbia clivicola* population.

The Radar Hill population of *Euphorbia clivicola* is fragmented by the constructed gravel road that traverses from west to east across the population (Figure 2.10). The distance between the two fragments is approximately 7 m; as such it will be regarded as two subpopulations (A is above the road and B is below the road) until further investigations to establish if meta-population principles apply on the two patches or not.

Figure 2.10 The locality of *Euphorbia clivicola* a) red triangle (above road), and b) green triangle (below road), divided by a gravel road at Radar Hill population in Polokwane.

1.2.2 Historical background

The city of Pietersburg was founded in the gold rush of the 1880's. The site of the city was initially owned by the Zuid-Afrikaanse Volksraad who purchased the eastern half of a large farm known as Sterkloop in 1884. The town was named after a well known General, Petrus (Piet) Jacobus Joubert. On the 31st of July 1886 Landdros (Magistrate) Dietlof Siegfred Maré wrote his first official letter from the magistrates' courts and the town was officially recognised. The main street through the city centre is known as "Landdros Maré Street" to this day. In February 2002, the city was renamed Polokwane - a Northern Sotho word that means "Place of Safety" (Polokwane Municipality, 2014).

1.2.3 Climate

The climate is characterised by summer rainfall with very dry winters. Polokwane receives about 462 mm of rain per year, with most rainfall occurring during summer. It receives the lowest rainfall (1.70 mm) in August and the highest (98 mm) in November (Figure 2.5). The 15 year summary reveals that the city received the least amount of rain in 2002 (256 mm) and the most in 2006 (678 mm) (Figure 2.6). The monthly average daily temperature for Polokwane ranges from a minimum of 5˚C in July to 29˚C in February (Figure 2.11) (South African Weather Service, 2013).

The city experiences higher wind velocity most of the year (Figure 2.7), with a mean daily minimum wind speed of 1.94 m/s observed in April and the maximum wind speed of 3.61 m/s occurring in October. The Polokwane metropolitan have a high percentage of humidity throughout the year with a monthly average of 73% and a mean daily minimum of 60% in September, and maximum of 81% in April (Figure 2.8) (South African Weather Service, 2013).

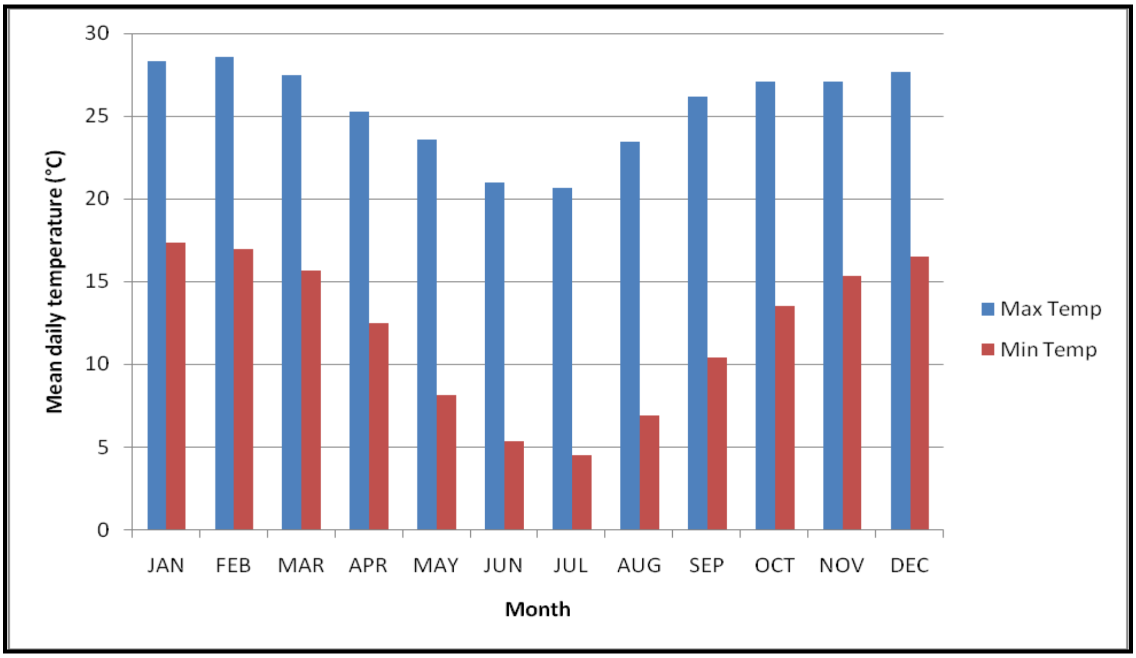


Figure 2.11 Mean minimum and maximum daily temperatures for Polokwane (1998 - 2012).

1.2.4 Geology

The geology and soils are characterised by Migmatites and the Turfloop Granite. Some ultramafic and mafic metavolcanics, quartzite and chlorite schist of the Pietersburg Group are also found (Pfab, 1997).

1.2.5 Vegetation

The vegetation is moderately undulating plains with a short open tree layer and a well-developed grass layer, to grass plains with occasional trees at higher altitudes. Hills and low mountains of Mamabolo Mountain Bushveld are embedded within this unit. This area falls within the Polokwane Plateau Bushveld situated at the higher-lying plains around Polokwane (Mucina and Rutherford, 2006).

Notable shrub-tree components that occur in the area include; (small trees) *Acacia caffra*, *Acacia tortilis* var. *heteracantha*, *Combretum molle*, *Peltophorum africana* (shrub), *Ziziphus mucronata*, *Vachellia karroo*, *Acacia burkei*, *Ficus burkei*, *Dichrostachys cinerea* subsp. *africana*, *Melia azedarach*, *Rhus* (*Searsia*) *pyroides* var. *pyroides*, *Ormocarpum trichocarpum*, *Mundulea sericea*, *Spirostachys africana*, *Clerodendrum glabrum*, *Elaeodendron transvaalense*, *Gymnosporia glaucophylla*, *Pappea capensis* and *Sclerocarya birrea* subsp. *africana* (Potgieter, unpublished data).

The forb component that occur in the area include the following; (forbs) *Amaranthus spinosus*, *Boophane disticha*, *Commelina africana* var. *africana*, *Commelina modesta*, *Commelina africana* var. *barberae*, *Crotalaria spartioides*, *Leonotis microphylla*, *Gymnosporia buxifolia*, *Senecio inaequidens*, *Senecio venosus*, *Cucumis myriocarpus*, *Euclea crispa* subsp. *crispa*, *Dicerocaryum eriocarpum*, *Rhynchosia nitens*, *Datura stramonium*, *Gnidia* sp. and *Raphionacme hirsute* (Potgieter, unpublished data).

The grass component includes: *Aristidia congesta, Cenchrus ciliaris, Cymbopogon pospischilii*, *Digitaria diggenalis*, *Digitaria eriantha*, *Eragrostis gummiflua*, *Eragrostis racemosa*, *Eragrostis superba*, *Imperata cylindrica, Melinus repens* subsp. *repens*, *Setaria sphacelata* var. *spacelata*, *Setaria verticillata*, *Stipagrostis uniplumis*, *Themeda triandra*, and *Urochloa mosambicens* (Potgieter, unpublished data).

1.3 Dikgale area, Kgwareng village

1.3.1 Location

The Dikgale area is on the Highveld Plateau, and is bounded in the south and south-east by the Strydpoort Mountains and in the east and north-east by the Wolkberg (Figure 2.12) (Mucina and Rutherford, 2006).

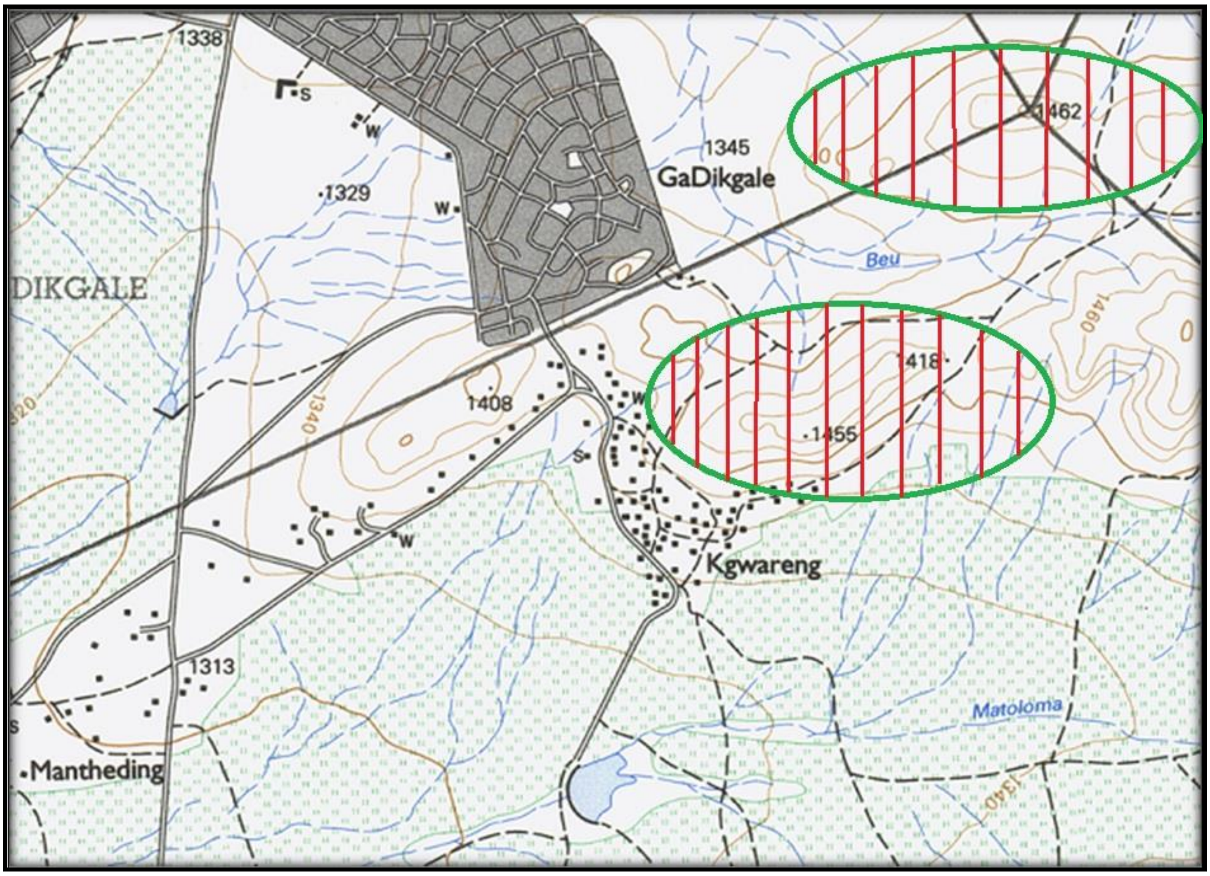


Figure 2.12 A 1:50 000 topographic map of Dikgale area. Chevroned area indicates the *Euphorbia clivicola* population.

The two subpopulations of *E. clivicola* at Dikgale are associated with short grasses and dwarf shrubs. A water reservoir was erected in 2012 (Potgieter, unpublished data) at Dikgale subpopulation A (Figure 2.13a). Another water reservoir exists at the Dikgale subpopulation B (Figure 2.13b).

a)

b)

Figure 2.13 Geographical and vegetation features of the localities the two subpopulations of Dikgale: a) Subpopulation A (south-facing), and b) Subpopulation B (north-facing).

1.3.2 Historical background

Geographically and culturally the Dikgale people belong to the Plateau North Sotho who are settled on the Highveld plateau in the vicinity of Polokwane. Shortly after 1700 they seceded from the Bakoni ba Matlala who live north-west of Polokwane. After a short stay with related Koni groups in Sekhukhuneland, they moved to settle in their present dwelling, an area about 50 km north-east of Polokwane. Here they conquered resident Venda groups, incorporated some of them as well as various families of North Ndebele origin. The cultural fusion that resulted from the assimilation of foreign groups distinguishes the Dikgale clan clearly from the pure Bapedi as well as the Lowveld tribe of the North Sotho (Rankoana, 2000). During the beginning of the post-apartheid era (1994), land claims were made by the Dikgale people to acquire more land.

The Dikgolo Trust is a group of people from the Dikgale tribal area, who came together to purchase land via the Settlement and Land Acquisition Grant (SLAG) of the Department of Land Affairs (DLA). Dikgale is in an area of relatively low rainfall, marginal for the production of rain-fed maize. Relatively high densities of human population and livestock mean that there are few opportunities for grazing in the area (Lahiff *et al*., 2008).

The first steps towards acquiring land for the people of Dikgale were taken by their traditional leader, Chief Dikgale, who, in 1996/97, encouraged all members of the community who were interested in acquiring land to register their names at the tribal authority office. In April 1998, the Nkuzi Development Association applied to the Community Facilitation and Support Fund of the DLA to facilitate the project to the point of approval of SLAG funding. In order to make the process more manageable, the membership was divided into four groups, who called themselves Dikgale, Dikgele, Dikgolo, and Dikgulu. All four Dikgale groups were interested in acquiring land adjacent to their tribal area, partially for convenience but also because they had a sense of historical claim on that land, having been forcibly dispossessed of it in the past. Portion 8 of Nooitgedacht farm was earmarked for the Dikgolo group, and other Dikgale groups targeted portions of the neighbouring Rietpol farm (Lahiff *et al.*, 2008).

1.3.3 Climate

The Dikgale area lies in a semi-arid to arid climatic type, with an annual rainfall of approximately 505 mm. It has a daily average summer temperature of between 17˚C and 28˚C. The winter average temperature range between 4˚C and 20˚C. Summer rainfall occurs between October and April, and then followed by a dry winter season (Rankoana, 2000).

1.3.4 Geology

Soils found in this area are related to the parent material which is granite (Matoks granite). Gritty sandy loam to sandy texture is found on the summit, back and shoulder slopes of the landscape. The valley bottoms have clay subsoil horizons, having tints of reddish mottles as an indication of a fluctuating water table. Soils found in this area are very poor in nutrients and erosion is high to moderate (Rankoana, 2000)

1.3.5 Vegetation

The area falls within the Polokwane Plateau Bushveld (Mucina and Rutherford, 2006). The area consists of moderately undulating plains with a short open tree layer with a well-developed grass layer to grass plains and occasional trees at higher altitudes. The area is least threatened, but with over one third of the remaining vegetation regarded as degraded, would probably be regarded as susceptible. Dense concentrations of human settlements are found in this area.

The vegetation is characterised by mixed bushveld. The area has a number of invasive species such as *Agave americana*, *Jacaranda mimosifolia*, *Melia azedarach*, *Opuntia ficus-indica* and *Ricinus communis* (Mucina and Rutherford, 2006). The two variations are the *Combretum apiculatum* veld that consist of small trees, quite dense and sometimes scrub-forest veld (Potgieter, unpublished data).

The shrub-tree component consists of 4 species which include: *Vachellia karroo*, *Dombeya rotundifolia*, *Gymnosporia heterophylla* and *Ozoroa sphaerocarpa*. The forb component contain 19 species, which include the following: *Aloe marlothii* subsp. *marlothii*, *Aloe greatheadii*, *Aptosimum procumbens* var. *elongatum*, *Avonia rhodesica*, *Carissa bispinosa*, *Cleome monophylla, Cotyledon orbiculata* var. *oblong*, *Crassula capitella*, *Conopus didymus*, *Dicerocaryum eriocarpum*, *Euphorbia shinzii*, *Euphorbia clivicola*, *Grewia vernicosa*, *Lopholaena coriifolia*, *Opuntia ficus-indica*, *Sarcostemma viminale*, *Senecio babertonicus*, *Xanthium spinosum* and *Ziziphus mucronata.* The grass component includes 8 species: *Aristidia congesta*, *Cynodon dactylon*, *Eragrostis tortilis*, *Eragrostic curvula*, *Melinus repens*, *Panicum maximum*, *Themeda triadra* and *Sporobolus africanus* (Potgieter, unpublished data).

1.4 Makgeng village, Boyne area

1.4.1 Location

The Boyne cluster is located in the south-western part of the Polokwane Local Municipality and includes the settlements of Boyne, Magokubung, Makgeng, Makgopeng, Mankgaile, Mountain View, Sobiaco, Viking, and Zion City Moria (Figure 2.14), falls within the Polokwane Plateau Bushveld situated at the higher-lying plains around Polokwane, north of the Strydpoort Mountains, with an altitude ranging from 1100 to 1500 m above sea level (Mucina and Rutherford, 2006).

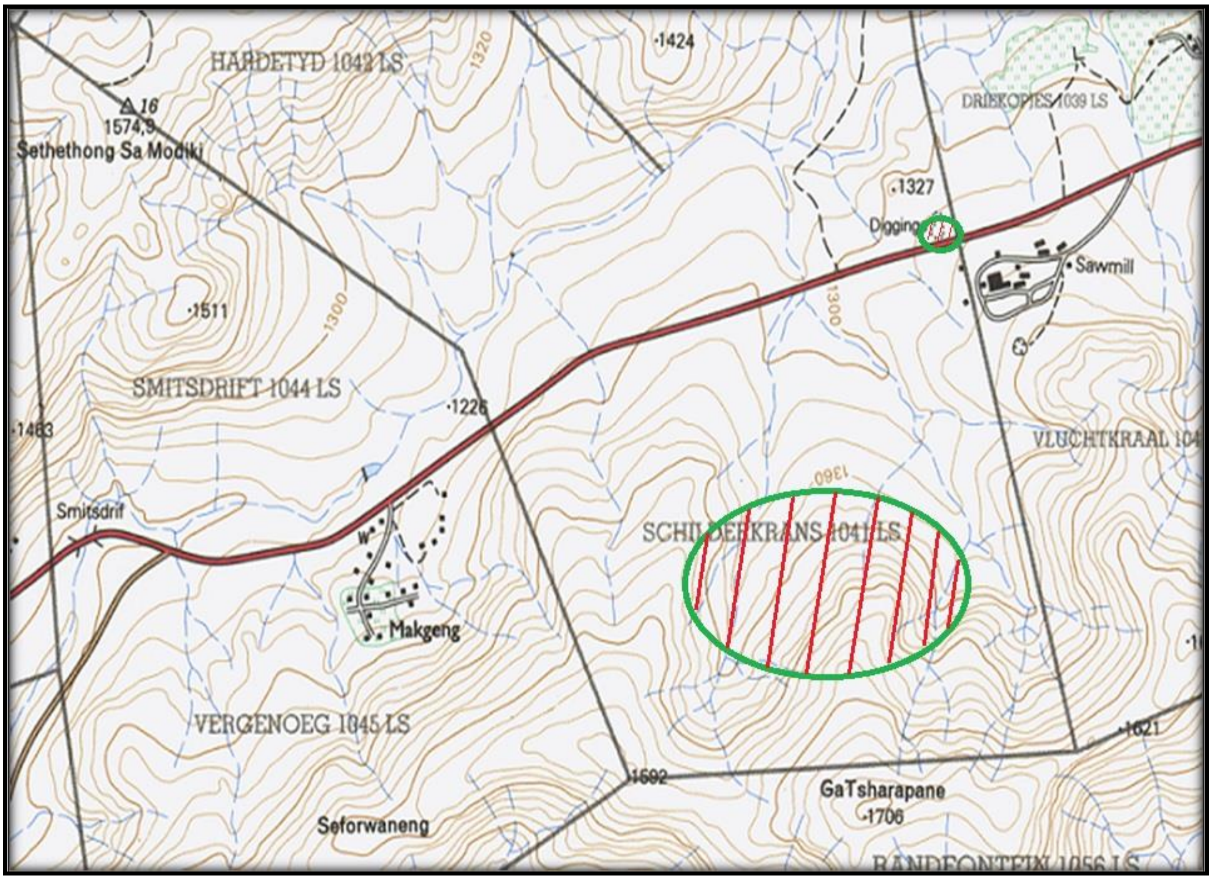
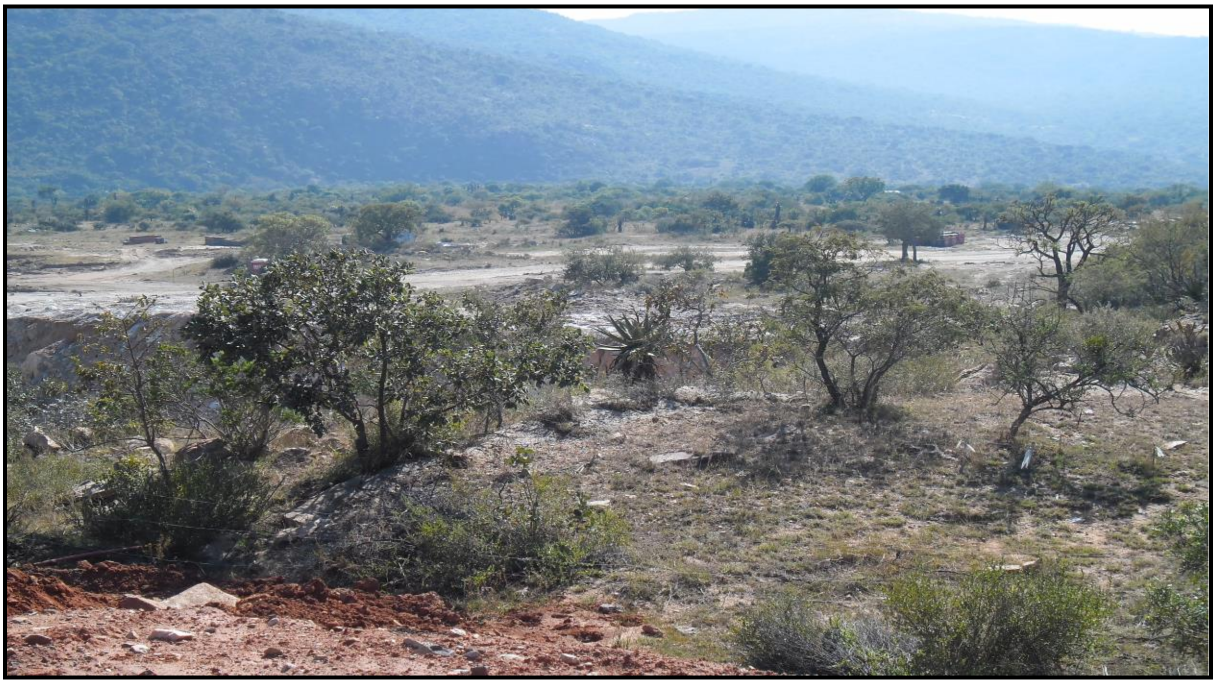


Figure 2.14 A 1:50 000 topographic map of Makgeng village. Chevroned area indicates the *Euphorbia clivicola* populations.

Two subpopulations of *Euphorbia* (*incertae sedis*) were discovered below (Figure 2.15.a) and above (Figure 2.15b) the R71 road next to Makgeng village. The subpopulation below the R71 road occurs next to quarry.

a)



b)

Figure 2.15 Geographical and vegetational features of the localities of the two subpopulations of *Euphorbia* (*incertae sedis*) at Makgeng that are divided by the R71 road: a) population below the R71 road (north-facing), and b) population above the R71 road (north-facing).

1.4.2 Historical background

No published information could be found on the history of Makgeng village.

1.4.3 Climate

The area is characterised by summer rainfall with very dry winters. Average monthly maximum and minimum temperatures for Polokwane is 29˚C and 5˚C for February and July, respectively.

1.4.4 Geology

Migmatites and gneisses of the Hout River Gneiss and the Turfloop Granite are dominant. Some ultramafic and mafic metavolcanics, quartzite, and chlorite schist of the Pietersburg Group are also found. Soils are variable, with freely drained soils containing high base status. There are also Glenrose and Mispah soil forms (Mucina and Rutherford, 2006).

1.4.5 Vegetation

The vegetation found in this area falls within the same vegetation type as that of Dikgale region and Radar Hill (Polokwane) and is characterised by short open tree layer, a well-developed grass layer to grass plains with occasional trees at higher altitudes (Mucina and Rutherford, 2006).

Trees and shrubs that occur in this area include; *Acacia caffra, A. permixta, A. rehmanniana, A. karroo, A. tortilis, Combretum molle, Ormocarpum kirkii* and *Ziziphus mucronata* (small trees)*. Aloe marlothii* subsp. *marlothii* (succulent tree)*. Acacia hebeclada* subsp. *hebeclada, Gymnosporia senegalensis* and *Lippia javanica* (tall shrubs) (Mucina and Rutherford, 2006).

2 MANAGEMENT

2.1 Percy Fyfe Nature Reserve

The reserve is managed by the Limpopo Department of Economic Development, Environment and Tourism (LEDET) and the Limpopo Tourism Agency (LTA). The two agencies are responsible for conservation and tourism management, respectively (Percy Fyfe Nature Reserve Strategic Plan, 2013). The main objective of the reserve is the breeding of rare antelope species for relocation purposes. The *E. clivicola* population was previously housed in the Tsessebe Camp (Pfab, 1997). Due to the impact of herbivory on this species, the antelope were relocated to another camp. A fire regime of three to five years burning intervals is also employed at the reserve to maintain a healthy ratio of grasses and trees (savanna vegetation), and in cases of drought it can be extended to eight year intervals.

2.2 Radar Hill

The Polokwane population occurs on municipal land which is ecologically unmanaged. A large proportion of the land is being used for residential purposes. Nearby residents also use the area for mountain biking. The vehicle and bike tracks have resulted in the division of the population into two subpopulations. According to the municipality (Polokwane Municipality, 2014) a motor city is to be erected at the northern side of the population during 2015.

2.3 Kgwareng and Makgeng Villages

The two sites are ecologically unmanaged and occur on grazing land for live-stock of the inhabitants.

CHAPTER 3

DNA ANALYSIS

1 INTRODUCTION

1.1 Threats to endemic species

According to the South African National Biodiversity Institute (2012), nearly a quarter of the South African Flora is considered either threatened with extinction or of conservation concern. As such, conservationists, in particular conservation biologists, are faced with the daunting task of identifying biological information necessary to evaluate the causes of threats, and ensuring the continued survival of the target species in nature (Schemske *et al*., 1994).

1.2 Anthropogenic habitat fragmentation

Anthropogenic habitat fragmentation is the breakup of a continuous habitat into several smaller spatially isolated remnants. These fragmentations are mostly caused by deforestation, urbanisation, and construction. Habitat fragmentation is a significant threat to the maintenance of biodiversity in many terrestrial ecosystems (Young *et al*., 1996). Small and isolated populations are more prone to demographic, environmental and genetic stochasticity, as well as allele and edge effects (Oostermeijer *et al*., 2003).

1.3 Genetic approach on conservation

During the last few decades there was a surge in academic debate between plant conservation biologists about the most appropriate way to approach a conservation problem; the so-called ecological vs the genetic approach (Escudero *et al*., 2003). Maintenance of genetic diversity is central to the fundamental goal of conservation biology, thereby preserving the evolutionary potential of native taxa. Genetic variation is thought to increase the probability of population persistence (Fleishman *et al*., 2001). Many naturally occurring species are subdivided into local demes, and gene flow between such populations counteracts the spatial genetic differentiation (Kumar *et al*., 2010). A species without an appropriate amount of genetic diversity is thought to be unable to cope with changing environments. Genetic bottlenecks and genetic drifts may result in many deleterious effects, not limited to inbreeding or outbreeding/outcrossing (Qian and Hong, 2001).

1.4 Inbreeding

Inbreeding, defined as mating between individuals that are more genetically similar than individuals drawn at random from the population, is known to reduce reproduction and survival of the offspring (Angeloni *et al*., 2011). By reducing heterozygosity within individuals, inbreeding alters the distribution of genetic variation, thus increasing the expression of recessive alleles and reducing the contribution of over-dominance (Carr and Eubanks, 2002). As such, the effects of inbreeding on herbivore resistance and exposure to pathogens can impact population dynamics in a variety of ways. Levels of inbreeding increase in small populations, and inbreeding depression for herbivore/pathogen resistance may further increase the risk of extinction in small populations (Stephenson *et al*., 2004). Some models that incorporate inbreeding depression predict the evolution of either complete selfing or complete outcrossing (Dolgin *et al*., 2007). In plants, the level of inbreeding depression is generally determined by comparing the effects of selfing and outcrossing on fitness, and often is defined as one minus the relative performance of selfed versus outcrossed progeny (van Treuren *et al*., 1993).

1.5 Outcrossing

Increased offspring number and increased offspring fitness via heterosis are often considered to be two of the chief benefits of outcrossing. Conversely, outcrossing can result in a fitness decline known as outcrossing depression (Sheridan and Karowe, 2000). Discussions of the causes of outbreeding have been divided into an ecological mechanism and a genetic mechanism of epistatic interactions between multilocus gene complexes. The ecological mechanism assumes that subpopulations are differentiated by the adaptation to different environments at microsites. In contrast the genetic mechanism assumes an identical environment in all populations and that restricted gene flow between and drift within populations by chance create co-adapted gene complexes by epistatic interactions (Schierup and Christiansen, 1996). A species without an appropriate amount of genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites (Qian and Hong, 2001). Identifying and distinguishing levels of genetic diversity in and among populations can be used to validate or dismiss claims of species relationships based on cladistics (Avise *et al*., 1987).

1.6 Inter Simple Sequence Repeats

There are various ways of analysing DNA diversity from different organisms. DNA techniques have gained ground, especially those based on the Polymerase Chain Reaction (PCR) such as microsatellites (or SSR), RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeat), and AFLP (Amplified Fragment Length Polymorphism), in part because these molecular markers provide a larger number of potentially polymorpic loci than allozymes (Escudero *et al*., 2003). Wu *et al*. (2010) noted that many studies, however, have shown that RAPD marker technique is not a robust and effective method for measuring variability and determining diversity because of its instability and poor reproducibility as compared to the ISSR marker system. An ISSR molecular marker technique permits the detection of polymorphism in microsatellites and inter-microsatellite loci without previous knowledge of DNA sequences (Ye *et al*., 2012).

2 RATIONALE OF THE RESEACH PROJECT

The identity of the *Euphorbia* species at Makgeng is uncertain. As such we address the species as *Euphorbia* (*incertae sedis*) (Figure 3.1), because this taxon does not match the morphological characteristics of *E. clivicola*.

Pfab (1997), alluded to the importance of a genetic diversity analysis for *Euphorbia clivicola* as a way of better understanding the relationship of individuals in a population, as well as the relationship between various populations. This investigation will serve as a baseline study for incorporating genetic diversity information in attempts to conserve *E. clivicola* in terms of transplantation.

Figure 3.1 *Euphorbia* (*incertae sedis*) from the Makgeng population.

3 RESEARCH QUESTIONS

a) What is the level of genetic difference between populations of *Euphorbia clivicola*?

b) What is the level of genetic variation between *Euphorbia clivicola* and *Euphorbia* (*incertae sedis*) using *Euphorbia schinzii* as an out-group?

4 SPECIFIC AIM AND OBJECTIVES

4.1 Specific aim

This chapter aimed to investigate the genetic relationship among all four populations of *Euphorbia clivicola* using the ISSR DNA fingerprinting technique.

4.2 Specific objectives

The objectives of this research were to:

a) Determine the DNA diversity between various populations of *Euphorbia clivicola* using Inter Simple Sequence Repeats. These results will aid in translocation and re-stocking of the species to strengthen declining populations.

b) Investigate genetic variations between *Euphorbia clivicola* and *Euphorbia* (*incertae sedis*). This data will help answer identity questions of the taxon occurring at Makgeng.

5 METHODOLOGY

5.1 Plant material collection

Four populations of *E. clivicola* from Percy Fyfe Nature Reserve, Radar Hill, Dikgale and Makgeng village of Limpopo Province, South Africa were surveyed and accessions representing the four populations were collected by walking across each population and in accordance to the method of Huang *et al*. (2010) at 10—20 m intervals depending on the area of occupancy, to avoid collecting the same clones. The accessions were stored in ice to maintain DNA stability and transported to the Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, for DNA isolation and analysis. The specimens were given unique codes (Table 3.1) to make the identification of samples simple.

Table 3.1 Locations of samples considered for genetic diversity analysis.

Population	**Location**	**Latitude**	**Longitude**	**Sample size**
PF	Percy Fyfe Nature Reserve	029°10.116	24°02.321	8
RH	Radar Hill (Polokwane)	029°30.125	23°53.711	11
DK	Dikgale (populations A and B)	029°47.957	23°46.136	15
DX	Dikgale popX	029°47.832	23°46.063	10
MK below	Makgeng below R71 road	029°50.552	23°55.935	5
MK above	Makgeng above R71 road	029°50.473	23°56.304	7
OG	Out-group (*Euphorbia schinzii*)	Hennops River	Gauteng P.	3

5.2 DNA extraction

A ZR plant/seed DNA Miniprep kit acquired from Inqaba Biotechnical Industries (Pty) Ltd - Pretoria, South Africa was used for the DNA extraction. Before starting with the extraction, Zymo-spin IV-HRC Filters (green tops) were prepared prior to use by: 1) snapping off the base, 2) inserting the filter into a collection tube, and 3) spinning in a microcentrifuge at exactly 8,000 x g for 3 minutes. A mass of 150 mg of finely cut or ground plant sample was added to a ZR BashingBead Lysis Tube followed by an addition of 750 µl Lysis Solution. The ZR BashingBead Lysis Tube was secured in a bead beater fitted with a 2 ml tube holder assembly and processed at maximum speed for 10 minutes. The Lysis Tube was then centrifuged in a microcentrifuge at ≥10,000 x g for one minute. A volume of 400 µl supernatant was transferred to a Zymo-Spin IV Spin Filter (orange top) in a collection tube and centrifuged at 7,000 rpm (7,000 x g) for one minute. To the filtrate in the collection tube a volume of 1,200 µl of Plant/Seed DNA Binding Buffer was added. From the mixture 800 µl was transferred to a Zymo-Spin IIC Column in a collection tube and centrifuged at 10,000 x g for one minute. The flow from the collection tube was discarded and the previous step was repeated. To the Zymo-Spin IIC Column 200 µl of DNA Pre-wash Buffer was added, the IIC Column was placed in a new collection tube and centrifuged at 10,000 x g for one minute. A volume of 500 µl Plant/Seed DNA wash Buffer was added to the Zymo-Spin IIC Column and centrifuged at 10,000 x g for one minute.

The Zymo-Spin IIC Column was transferred to a clean 1.5 ml microcentrifuge tube and 50-100 µl (25 µl minimum) DNA Elution Buffer was added directly to the column matrix and centrifuged at 10,000 x g for 30 seconds to elude the DNA. The eluted DNA from the previous step was transferred to Zymo-Spin IV-HRC Spin Filter (green top) in a clean 1.5 ml microcentrifuge tube and centrifuged at exactly 8,000 x g for one minute. The filtered DNA was now suitable for PCR and other downstream applications.

5.3 Quantifying DNA

The determination of DNA quantity and quality was undertaken utilising two methods, spectrophotometric measurements (CARY 1E UV-visible) and agarose gel electrophoresis. Using the CARY 1E UV-visible spectrophotometer for each sample, 5 µl of DNA was pipetted and mixed with 195 µl of TE buffer in a 1 cm or 1 ml quartz microcuvette. Absorbance was read at 260 and 280 nm against a TE buffer blank. DNA quality was calculated by converting optical density (OD) reading to µg/ml (reading of 1.0 at OD_{260} is equivalent to 50 µg of DNA/ml). The DNA samples were further ran on 0.8% agarose gel electrophoresis to check the quality.

5.4 Inter Simple Sequence Repeat Analysis

Table 3.2 refers to the materials and concentrations used for the Polymerase Chain Reaction using the Type-it Microsatellite.

Table 3.2 Composition of the Type-it Microsatellite kit used for the Polymerase Chain Reaction.

Components	**Volume/reaction**	**Final concentration**
Reaction mix 2x Type-it Multiplex PCR Master Mix	12.5 µl	1x
10x primer mix (2 µM of each primer)	2.5 µl	0.2 µM of each primer
RNase-free water	Variable	-

Optional: 5x Q-Solution	5 µl	1x
Template DNA (added at step 4)	Variable	≤200 ng DNA/reaction start with 10 ng
Total reaction volume	25 µl	

5.5 Polymerase Chain Reaction conditions

Table 3.3 illustrates conditions used to facilitate the Polymerase Chain Reaction.

Table 3.3 Polymerase Chain Reaction cycling conditions outlined on the Type-it Microsatellite Polymerase Chain Reaction kit.

Step	**Time**	**Temperature**	**Comment**
Initial heat activation	5 min	95˚C	Activates HotStar Taq and DNA polymerase
3-step cycling: Denaturation	30 sec	95˚C	
Annealing	90 sec	32˚C	
Extension	30˚C	72˚C	Optimal for targets up to 0.5 kb in length
Number of cycles	35		The optimal number of cycles depends on the amount of template DNA and the required sensitivity of your detection method.
Final extension	35	60˚C	

5.6 Data analysis

ISSR amplified bands were analysed by scoring the presence as (1) and absence was scored as (0), and a binary qualitative data matrix was constructed. The matrix was constructed using GenAIEx version 6.41 program and exported to POPGEN version 3.2 program. The following parameters were generated using the program POPGENE 3.2 to describe inter-population genetic variation: observed heterozygosity (H_o), and the effective number of alleles (N_e) (Chen *et al*., 2009). Nei's (1978) gene diversity (*h*) using corrected allele frequency and the Shannon's index (*I*) were estimated among the populations. A UPGMA dendrogram was constructed based on Nei (1978)'s genetic distance, a method that was modified from NEIGHBOR procedure of PHYLIP version 3.5.

6 RESULTS

6.1 Inter Simple Sequence Repeat profile

Twenty one individuals from 6 natural populations of *Euphorbia clivicola* and 1 from *Euphorbia schinzii* (out-group) were amplified with one primer. A total of 84 clear and reproducible bands were produced with different lengths, of which 27 (33.33%) were polymorphic and 57 (66.67%) were monomorphic. The number of bands varied between 3 and 6, and averaged to 4 bands per sample (Figure 3.3).

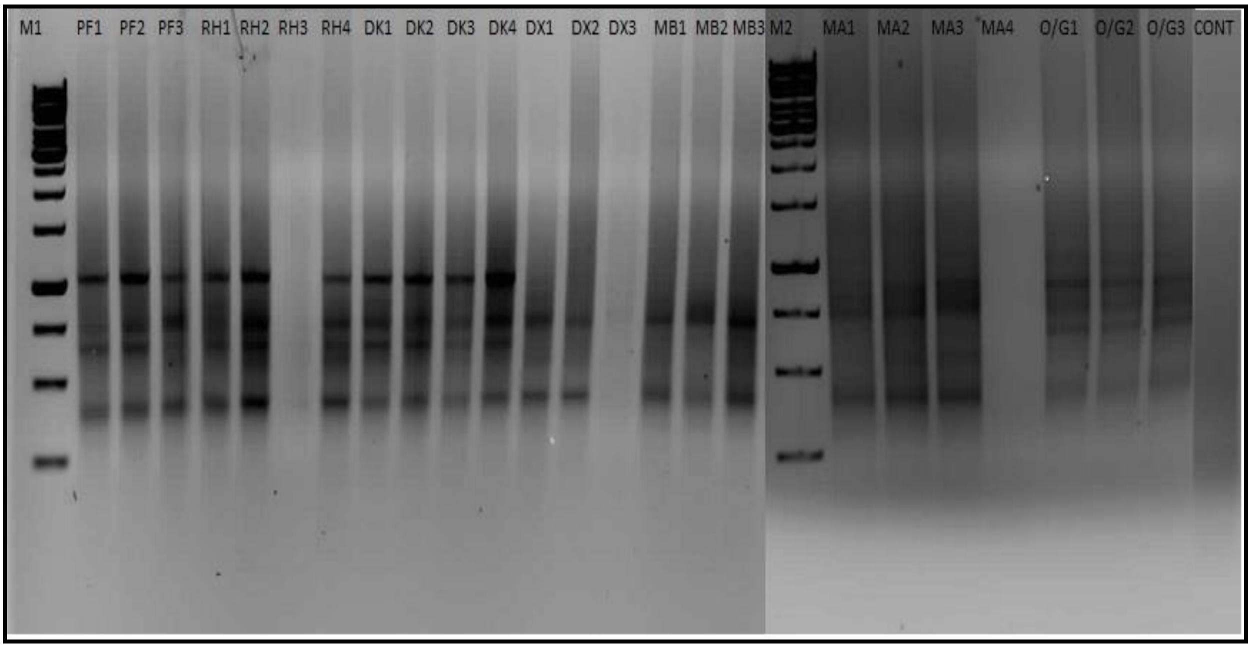


Figure 3.2 Electrophoresis pattern amplified from *Euphorbia clivicola* in Limpopo Province with primer ISSR861. Lane M1 and M2, GeneRuler 1kb DNA ladder, lane CONT represent a negative control; other lanes represent accessions from species listed in Table 3.1.

6.2 Inter population genetic diversity

The Nei's genetic diversity (*h*) and Shannon's index (*I*) between all the populations analyzed were estimated at 0.1088 (SD = 0.1763) and 0.1681 (SD = 0.2671), respectively. Genetic variations between the populations of Makgeng, Dikgale, Radar Hill, and Percy Fyfe revealed a low genetic diversity (*h*) of 0.0741 (SD = 0.1814) and Shannon's index (*I*) was 0.1061 (SD = 0.2599). From the total genetic diversity, 33.33% polymorphism was attributed to intra-population diversity, while 66.67% monomorphism was observed inter-populations. Genetic polymorphism between all populations examined was 16.67%, and showed a high (83.33%) genetic similarity. When comparing genetic diversity between *E. schinzii* (out-group), Makgeng (below and above the R71 road), and Dikgale popX, a genetic difference of (18.23%) was observed.

6.3 Genetic distance and relationships

The genetic distances, based on the allele frequencies of the ISSR marker were calculated for each pair of populations to estimate the extent of their divergence (Table 3.4). The average genetic distance between populations equalled 0.1514. The genetic distance between 7 populations ranged between 0.0000 and 0.4055, and the genetic identities were from 0.6667 to 1.0000. No genetic difference was observed between populations of Percy Fyfe Nature Reserve, Radar Hill, and Dikgale popX. A genetic distance was observed between the latter populations and the Makgeng below and above R71 road to be 18.23%. The greatest genetic difference was observed between *E. schinzii* and *Euphorbia* (*incertae sedis*) (40.55%).

A dendrogram (Figure 3.4) was obtained based on Nei's (1978) genetic distance using the UPGMA method, which was modified from the NEIGHBOR procedure of PHYLIP version 3.5. The UPGMA cluster analysis revealed clear genetic relationships among all the populations sampled. The populations were divided into two main clades: 1) Percy Fyfe Nature Reserve, Radar Hill, Dikgale, and Dikgale popX, and 2) Makgeng below and above R71 road, while *E. schinzii* stood out on its own.

Table 3.4 Genetic distance and geographical distances of *Euphorbia clivicola* and *Euphorbia schinzii* populations in Limpopo Province.

POPULATION	PF	RH	DK	DKX	MB	MA	O/G
PF	****	1.0000	1.0000	1.0000	0.8333	0.8333	0.8333
RH	0.0000	****	1.0000	1.0000	0.8333	0.8333	0.8333
DK	0.0000	0.0000	****	1.0000	0.8333	0.8333	0.8333
DKX	0.0000	0.0000	0.0000	****	0.8333	1.0000	0.8333
MB	0.1823	0.1823	0.1823	0.1823	****	1.0000	0.6667
MA	0.1823	0.1823	0.1823	0.1823	0.0000	****	0.6667
O/G	0.1823	0.1823	0.1823	0.1823	0.4055	0.4055	***

* Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

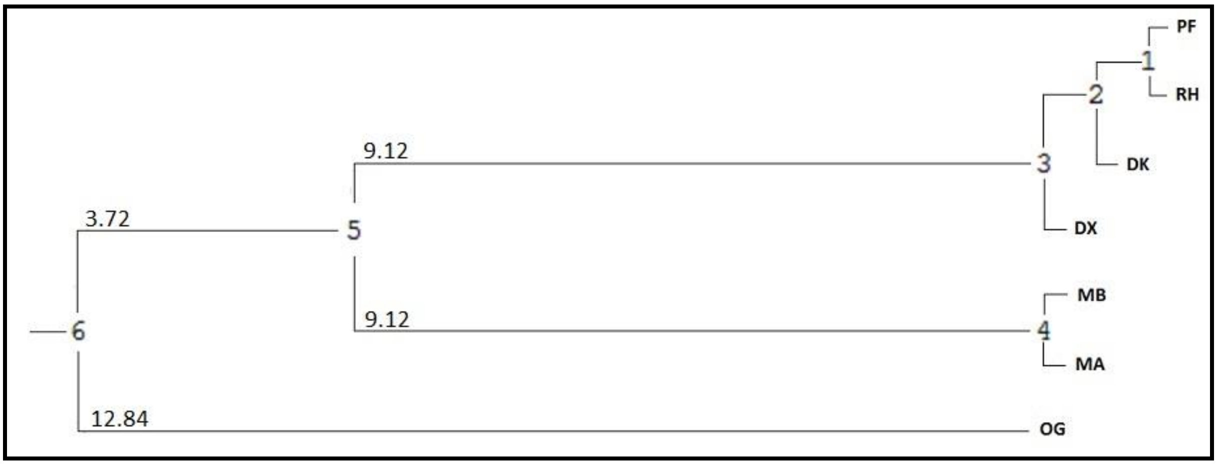


Figure 3.3 Dendrogram obtained by UPGMA cluster analysis based on Nei's genetic distance among the 7 populations (6 from *Euphorbia clivicola* and 1 from *Euphorbia schinzii* (out-group)). Abbreviations are given in Table 3.1.

7 DISCUSSION

This study is the first to assess the genetic diversity amongst populations of *Euphorbia clivicola* in comparison with *Euphorbia schinzii* (out-group). By using an ISSR marker, this study was able to reveal the genetic variations among *E. clivicola* populations and *Euphorbia* (*incertae sedis*) from Makgeng population whose identity was uncertain. The research questions for this chapter (three) were therefore answered.

The genetic variation between *E. schinzii* and Percy Fyfe Nature Reserve, Radar Hill, Dikgale, and Dikgale PopX populations was observed to be low (18.23%, h = 0.8333). No genetic variations were observed between populations of Percy Fyfe Nature Reserve, Radar Hill, Dikgale, and Dikgale PopX (Chapter 3, Section 3.a). Low genetic variation of *Murraya koenigii* was described by Verma and Rana (2011), and it was observed that the patterns of genetic variation detected among the populations of *M. koenigii* revealed that the gene flow among populations might be significant to prevent genetic drift indicating exchange of genes among them. This phenomenon implies that; the populations of *E. clivicola* (Percy Fyfe Nature reserve, Radar Hill and Dikgale) are genetically compatible for relocation during recovery plans.

In accordance with Figure 3.4, the UPGMA Dendrogram constructed using POPGEN version 3.2 program based on Nei (1978)'s genetic distance also revealed the genetic relationships between all the populations under study by grouping the populations into two clades. The clades grouped the populations according to their genetic similarities. This give rise to two revelations; a) The two previously known populations are genetically similar to the Dikgale population, and b) The *Euphorbia* (*incertae sedis*) is a variety of *E. clivicola* because of the 18.23% genetic variability between them. To consider the *Euphorbia* (*incertae sedis*) as a new species based only at the genetic analysis would be premature, as the classic classification system (cladistics) based on the morphological characteristics should be incorporated. It might also be the same species, but because of the geographical distribution they may have started to evolve different traits.

The 18.23% polymorphism between *E. schinzii* and *E. clivicola* (Percy Fyfe Nature Reserve, Radar Hill, Dikgale, and Dikgale PopX) populations indicate low genetic differences between the two species. These low genetic variations may imply that the two species have a common ancestor and they may be unable to evolve (adapt to the changing environment (Verma and Rana, 2011). *Euphorbia* (*Incertae sedis*) (Makgeng below and above R71 road populations) and *E. schinzii* showed a greater polymorphism at 40.55% between them. The 40.55% polymorphism means these are two different taxa. The (18.23%) genetic difference between the Makgeng subpopulations and the rest of the *E. clivicola* populations indicates a possible variety of the species under study; meaning a more in-depth taxonomic study is required. The above polymorphic percentages also indicate that the two Makgeng populations are more closely related to *E. clivicola* than to *E. schinzii*.

8 CONSERVATION IMPLICATIONS

Genetic diversity is critically important for a species to maintain its evolutionary potential to cope with an ever changing environment; loss of genetic diversity is often associated with reduced fitness. The maintenance of genetic variation is a major objective of conservation plans for endangered species (Chen *et al*., 2009). Information generated in this study provides baseline data on population genetics, which can contribute to conservation plans or be used to optimize *in situ* conservation via relocation or reintroducing genetically similar species to declining populations like Percy Fyfe Nature Reserve population (8 individuals).

Percy Fyfe Nature Reserve, Radar Hill, and the Dikgale populations are genetically similar; as such individuals can be intra-relocated among the above mentioned populations. Makgeng below and above R71 road are two special subpopulations because they differ genetically from the other populations of *E. clivicola*, as well as from the out-group. The Makgeng subpopulations might be a variety of *E. clivicola*; this indicated by the low genetic diversity between the two species. A more detailed taxonomic and genetic investigation is needed to morphologically and genetically characterize the Makgeng species.

This study was able to achieve its two objectives by: a) grouping the populations according to their genetic similarities, thus, aiding conservationist in avoiding an expensive mistake of reinforcing Percy Fyfe Nature Reserve population with genetically incompatible *E. clivicola* individuals, and b) genetically distinguishing the species at Makgeng subpopulations from *E. clivicola*. To evaluate the level of genetic diversity inter-populations more markers must be used to obtain the average number of effective alleles.

CHAPTER 4

THE EFFECT OF ABIOTIC FACTORS ON POPULATION BIOLOGY AND ECOLOGY

1 INTRODUCTION

1.1 Abiotic factors

The disappearance of 87% of the unprotected population (Radar Hill) and 91% of the protected population (Percy Fyfe Nature Reserve) between 1986 and 1997 (Pfab, 1997), is caused by a complex set of environmental factors. Environmental factors can be subdivided into abiotic (e.g. fire, soil, topography, climate) and biotic factors (e.g. herbivory, competition, anthropogenic activities), the latter will be investigated in the next chapter.

Abiotic factors, such as climate-driven responses to elevation (Diaz *et al.,* 1999) and soil properties (Baudena *et al.,* 2013), can influence which plant species will colonize a site (co-existence and competition). This is because species differ in their tolerance and utilization of resources (Laughlin and Abella, 2007). As such, it can be said that the effects of abiotic factors influence biotic factors, which collectively have an effect on the population biology (e.g. development stages, canopy size, pollination syndrome, flowering and fruiting) and ecology (e.g. density, dispersal, and demographics) (Diaz *et al.,* 1999). The link between abiotic-biotic factors and population biology can be described as population ecology.

1.2 Life stages

According to Van Tonder (2011), there are four developmental stages of a plant that are of utmost importance to plant ecologists and biologists. These are: seedling, juvenile, adult and senescent. Determining the cause of the decline of a species is not simply a question of which factor is threatening the species' existence, but also which life stage is being affected (Österling *et al.,* 2008).

Fire promotes the recruitment of seedlings by decreasing intra- and inter-specific competition when it temporarily eliminates grass and weakens other competitors, and increases the amount of light and water availability (Diadema *et al.,* 2007). Fire further accelerates the decomposition of matter by incineration, subsequently increasing macro and micro-nutrients available in the soil. In the case of *E. clivicola,* Pfab and Witkowski (1999a) reported the ability of plants to resprout after they experienced fire damages of 40%—89%. Thus, their canopy cover will significantly increase due to the new growth coupled with post fire remnants.

1.3 Soil chemistry

Optimal growth and development of plants depend on various environmental factors. Among them the mineral nutrients available in the soil play an important role (Iqbal *et al*., 2013). Soils from the two formerly known populations (Percy Fyfe Nature Reserve and Polokwane populations) of *E. clivicola* were found to be acidic, with the pH ranging between 4.71—4.78, and various heavy metals were also detected (Pfab, 1997). Heavy metals are defined as a group of elements that have a density higher than 5mg/cm (Hamid *et al*., 2010). Heavy metal pollution of soil and water is a very serious environmental problem, with potentially harmful consequences for the ecosystem (Aghaee *et al*., 2013). High concentrations of heavy metals are toxic and affect the growth of plants, and also induce alteration in seed germination potential. In most terrestrial ecosystems, there are two main sources of heavy metals: Natural sources (e.g. parent material, volcanoes, continental dust, rock weathering) and anthropogenic sources (e.g. extraction companies, industrialisation, and urbanisation) (Khan *et al*., 2014).

1.4 Seeds dispersal

According to Pfab (1997), *E. clivicola* disperses its seeds ballistically within an estimated distance of < 1 m. Pfab (1997) further alluded to the secondary distribution mechanism induced by steep slopes that suggests downhill dispersal from the parent. The strong influence of slope angle on seed dispersal was further advocated by Cerda and Garcia-Fayos (1997), where they investigated the rate of seed dispersal on slopes and it was evident that seed moved in a downslope direction.

2 RATIONALE

Due to the stationary character of plants, they undergo continuous exposure to various abiotic stresses in their natural environment (Fujita *et al*., 2006). It was hypothesised by Laughlin and Abella (2007) that most abiotic stresses have reciprocal relations with biotic stressors. Once plant species passes through the abiotic filters, the remaining species undergo further filtering by biotic interactions. The rationale for this chapter was to investigate the abiotic agents that led to the decline of *E. clivicola* populations at Percy Fyfe Nature Reserve and Radar Hill, as well as the factors in play at Dikgale and Makgeng that affect the biology and ecology of their subpopulations. These will be achieved by relating various abiotic features such as fire, slope aspect, and soil chemistry to the biology (population structure, density, canopy size, flowering, fruiting and dispersal), and ecology of *E. clivicola*.

Our aim was to gain insight into the relationship between abiotic features and the biology and ecology of *E. clivicola*. Findings from this investigation will aid officials from various conservation agencies in the Limpopo Province to make informed decisions when implementing the proposed Adaptive Management Plan (Chapter 6) of this critically endangered species.

3 RESEARCH QUESTIONS

a) What are the current population sizes class distribution of all known populations of *Euphorbia clivicola* and *Euphorbia* (*incertae sedis*)?

b) What are the effects of fire on *Euphorbia clivicola*'s biology and ecology?

c) What are the implications of various features (ground, stone and rock) cover on *Euphorbia clivicola*?

d) How does slope angle and aspect influence seed dispersal and expansion direction *Euphorbia clivicola*?

4 SPECIFIC AIM AND OBJECTIVES

4.1 Specific aim

The specific aim of this chapter was to determine the effects of abiotic factors on the population biology and ecology of *Euphorbia clivicola*.

4.2 Specific objectives

This chapter determined the:

a) Demography of the four populations of *Euphorbia clivicola*. These data will give insight into the population dynamics of *Euphorbia clivicola*.

b) Effects of fire on the biology and ecology of *Euphorbia clivicola*. The data will aid in valididating Pfab's (1997) recommendations on the fire regime.

c) Influence of abiotic features cover on the biology and ecology of *Euphorbia clivicola*. These data will aid conservationists on which attribute of the habitat to manage and monitor for the persistence of *Euphorbia clivicola in situ*.

d) Effects of slope and aspect on the biology and ecology of *Euphorbia clivicola*. These will aid conservationists on the habitat requirements of *Euphorbia clivicola* and the influence slope has on seed dispersal.

e) Effects of soil minerals on the biology and ecology of *Euphorbia clivicola*. The information gathered will also aid tissue culturists on the nutrient chemistry *Euphorbia clivicola* prefers.

5 METHODOLOGY

5.1 Population biology

5.1.1 Area of occupancy

The area of occupancy (AOO) of each population was determined by using a global positioning system (GPS), and by including as many GPS coordinates on the population's perimeter as possible. This was determined by walking from the approximate centre of the population's distribution outwards, until the last plant was found. The centre of the population was determined during the preliminary site visit, by walking the entire Area of Occupancy. It was repeated until the perimeter, and hence the AOO of each population was established. The GPS coordinates were then superimposed onto a Google Earth image (Google Earth, 2015), and a Polygon was created and measured (AOO) (Figure 4.7).

5.1.2 Population demography

Sampling techniques were chosen considering the sizes of the area of occupancy. The two smaller populations of Percy Fyfe Nature Reserve and Radar Hill were sampled using the Nearest Individual Sampling Technique (NIST). The NIST entails that the plant nearest to the sample point is located (plant). The distance between it and the sample point is then measured. The density is thus calculated as follows:

Density = $1/(2D_m)^2$

Where D_m = mean distance for all samples (Hill *et al.*, 2006).

The populations from Radar Hill, Dikgale and Makgeng, were divided into subpopulations because of the patchy AOO. Hence the Radar Hill and Makgeng (below R71 road) populations were small. Populations with a greater AOO were sampled using the Point Centre-Quarter method (PCQ).

The PCQ method (Figure 4.1) was employed on all the remaining (large) populations. This method was used in combination with transects laid across the population. The PCQ method involves two perpendicular straight lines crossing each other at a sample point (Mark and Esler, 1970). In this way four quadrants are created by the straight lines crossing each other forming the centre of all four quadrats (Hill *et al.*, 2006). The distances from the central point to the nearest *E. clivicola* plant in each quadrant (e.g. A, B, C and D) is then measured (Hill *et al.*, 2006). The distances are then averaged, and density calculated. The density is calculated as:

Density = $1/D_m^2$

Where D_m = mean of average distances (Hill *et al.*, 2006).

These transects were laid out parallel to one another in an east−west (along the hill top) and north−south (from the foot hill to the top) direction. Transects ran from one end (on the boundary) of the population in a straight line to the other end (boundary) of the population. All transects covered 25—30% or more of each respective population. Each transect differed in length (from 30 m to 600 m) in relation to the AOO of each population. Knots were made in the rope at 5 m intervals, representing the point-centre of each 5 m x 5 m quadrat. Thus, a continuous string of quadrats where placed along the length of a transect. The distance between transects were 10 m apart. Each transect was traversed by two people who systematically searched for nearest plants from the centre point on either side of the transect line in each quadrat. Due to the large areas occupied by populations, areas of variable densities were encountered. To ensure consistency, one plant was measured in terms of its canopy area, in the last quadrat of each set of four quadrants (quadrat D). In a case where there was no plants encountered in one of the four 5 m x 5 m quadrats; we searched beyond the 5 m x 5 m perimeter. We made sure that the sampling point lines where our borders, which meant the quadrants were not closed at the extreme ends of the transect (Figure 4.1).

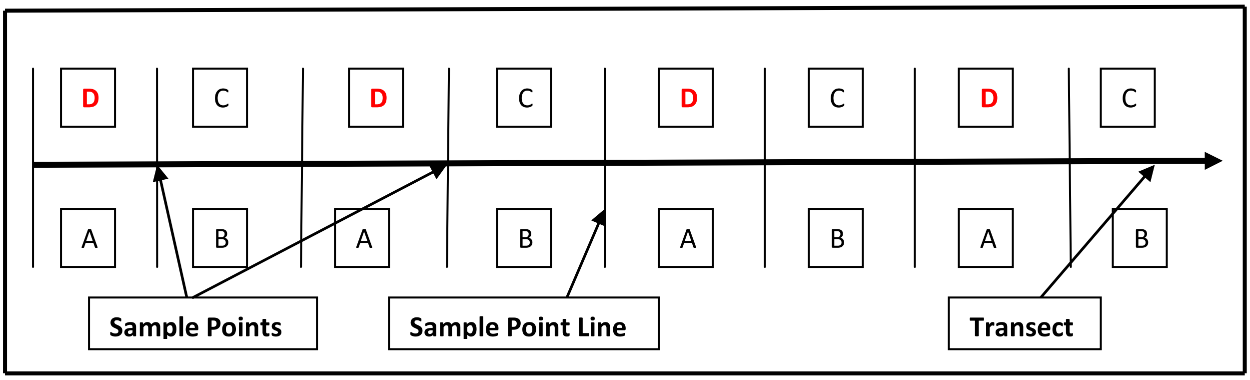


Figure 4.1 An illustration of the Point Centre-Quarter method.

5.1.3 Life stages and canopy size

All located and sampled plants for all populations were classified into one of the following four classes:

a) Senescent plants

Plants that showed no signs of life (Pfab, 1997) (Figure 4.2a).

b) Adults

Plants showing true flower formation or definite positive signs of flower formation (buds). Adult plants are characterised by a large underground tuberous body and many above-ground branches (2—3 cm in length) congested together in a dense mass (Pfab, 1997) (Figure 4.2b).

c) Juvenile plants

Plants showing either primary or secondary growth characteristics, but flower formation or signs of flower formation is absent. Juvenile plants have a poorly-developed underground tuberous body, and form two or a few small loosely arranged above-ground branches (Pfab, 1997) (Figure 4.2c).

d) Seedlings

Seedling plants having only one above-ground branch (Pfab, 1997) (Figure 4.2d).

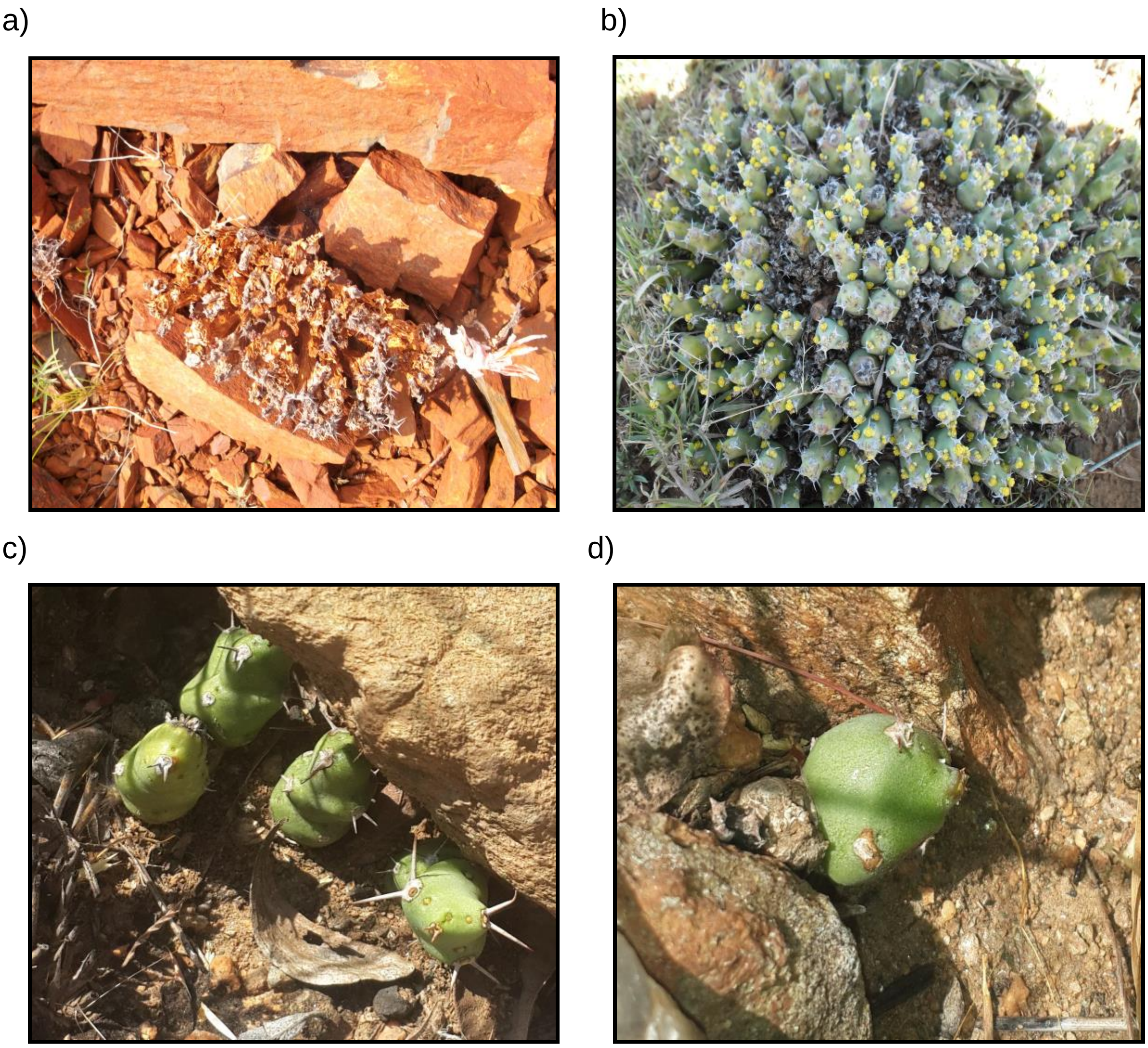


Figure 4.2 Four age classes of *Euphorbia clivicola*; a) senescent, b) adult, c) juvenile, and d) seedling.

The size of each plant sampled from all known populations was recorded. Where (W_1) was the widest diameter of area covered by the plant and (W_2) was the diameter perpendicular to first one. The readings were subsequently used to calculate the canopy area using the following equation (Pfab, 1997):

$$\text{Canopy area} = \pi \times L_1/2 \times L_2/2$$
$$= 0.7854 \times L_1 \times L_2$$

5.1.4 Recruitment capacity

The number of flowers and fruits where recorded for each plant sampled at all four populations. These results aims to show the recruitment capacity of *E. clivicola* and the presence of pollinators that transforms flowers into fruits through pollination. Various plants per population were visited several times during the flowering and fruiting season of 2013.

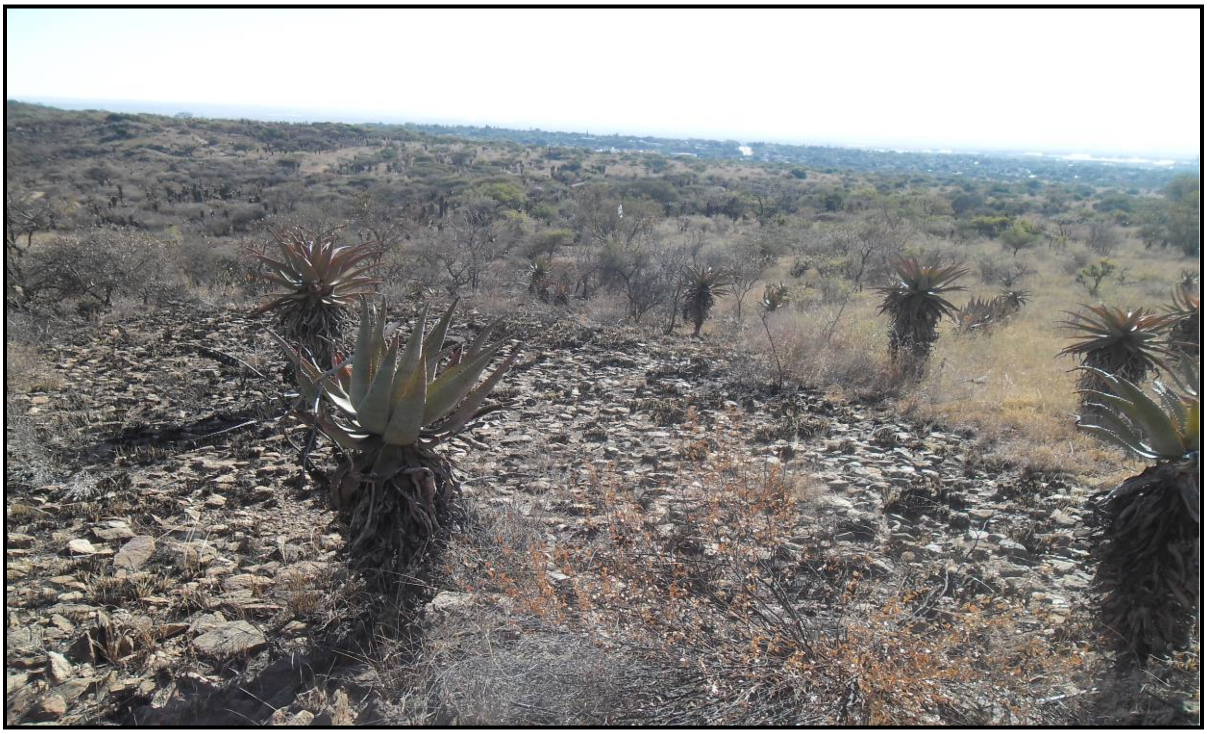
5.2 Abiotic factors

5.2.1 Fire

After an accidental field fire in August 2013 at Radar Hill had occurred, an opportunity to challenge or validate Pfab and Witkowski's (1999a) findings and recommendations regarding the proposed fire regime on *E. clivicola* was utilised. The following sampling method was used:

From September 2013 to May 2014, all vegetation response to fire were analysed in comparison with vegetation structure that was un-burnt. Three permanent plots, two burnt (Figure 4.3a) and one un-burnt (Figure 4.3b) of 10 m x 10 m were laid out 5 m apart on the north-facing slope of the upper subpopulation of the peri-urban population (Figure 4.3). Total species count in each plot was conducted by two people traversing the plots. Fifty-three plants where identified in the two burnt plots and 31 in the un-burnt plot. A unique number was assigned to each individual in all the plots. All measurements were recorded via a data sheet (Appendix 1.1) on a monthly basis to insure that the rates of regeneration and recruitment post-fire were accurate.

a)



b)

Figure 4.3 A 10 m x 10 m plot at Radar Hill population; a) burnt plot, and b) un-burnt plot.

5.2.2 Quantifying fire damage

In quantifying fire damage, three classes of branch damage were defined. These are: wilted (Figure 4.4), incinerated (Figure 4.5), and incinerated senescent (Figure 4.6) damage.

a) Wilted-Top/tip of the branch is scorched by fire, a brown or black colour appears while the base of the branch is still green (Figure 4.4).

Figure 4.4 Wilted branch of *Euphorbia clivicola* from the burnt plot at Radar Hill population.

b) Incineration of living branches - were characterised by the whole branch being completely burnt, with brown-coloured branches.

Figure 4.5 Incinerated living branches of *Euphorbia clivicola* from Radar Hill population.

c) Incinerated senescent branches - these are burnt branches showing a black colour. This damage was not considered as being caused by fire because the branches were already senescent prior to the fire (Figure 4.6).

Figure 4.6 Incinerated senescent branches of *Euphorbia clivicola* at Radar Hill population.

5.2.3 Measuring vegetative vigour and recruitment

Regrowth was quantified by the percentage of new growth, where total number of branches (damaged and un-damaged) were counted against the number of new branches (young). Demographic and canopy size data were collected according to Pfab (1997). Flowering and fruiting ratio was also recorded in both burnt and un-burnt plots. Density was noted in metres (m) as the distance from an individual plant to the nearest neighbour.

5.3 Soil analysis

5.3.1 Collection

Soil samples were taken from 0.4 m x 0.4 m quadrats in selected areas in each population as follows: 5 samples per subpopulation, at the north, south, east, west corners and at the centre; hence there were 4 subpopulations and thus 20 samples. Approximately 2 kg per sample was collected. Samples were taken to the Department of Soil Science, University of Limpopo for analysis. Each sample was air-dried on laboratory benches for three days, and then sieved through a 2 mm sieve. The sieved soil (<2 mm fraction) was packed into two separate boxes. The depth of the soil sample taken may have varied according to local conditions, e.g. a rock or sandy substrate, but did not exceed 60 cm.

After the soil was air-dried for three days, the samples were analysed in the laboratory using the Handbook of Standard Soil Testing Methods for Advisory Purposes (1990).

5.3.2 Particle size (Hydrometer Method)

The samples were treated with sodium hexametaphosphate to complex Ca^{++}, Al^{3+}, Fe^{3+}, and other cations that bind clay and silt particles into aggregates. Organic matter was suspended in this solution. The density of the soil suspension was determined with a hydrometer calibrated to read in grams of solids per litre after the sand settles out and again after the silt settles. Corrections were made for the density and temperature of the dispersing solution.

5.3.3 Extractable cations: Ammonium acetate (1 mol dm^{-3}, pH 7)

5 ± 0.05 g air-dried, ≤ 2 mm soil were placed in 100 cm^3 extraction bottle. Fifty cm^3 NH_4OAc solution cooled to 20 ± 2ºC to the soil in the extraction bottle and shaken horizontally on a reciprocating shaker at 180 oscillations per minute for 30 minutes. The solution was rapidly filter extracted through a Buchner funnel, with suction and filtrates collected, but the first few drops were discarded, and re-filtered if the extract was not clear. The elements K, Na, Ca and Mg in the filtrate were determined by flame emission spectroscopy.

5.3.4 Organic carbon: Walkley-Black

Iron (II) ammonium sulphate solution was standardised daily against 10 cm^3 0.167 mol dm^{-3} $K_2Cr_2O_7$. Soil was ground to pass a 0.35 mm sieve, using a porcelain mortar and pestle. A weight of 1 g (0.5 g or less if high in organic carbon) of air dried soil was added to a 500 cm^3 Erlenmeyer flask. A volume of 10 cm^3 of $K_2Cr_2O_7$ solution was added to the soil sample via a pipette. The flask was shaken to disperse the soil in the solution. A volume of 20 cm^3 concentrated sulphuric acid was rapidly added, and the stream was directed into the solution. The flask was again swirled gently until soil and reagents were mixed, then more vigorously for a total time of 1 minute. The flask was allowed to cool on a sheet of asbestos for 30 minutes. A volume of 150 cm^3 de-ionised water and 10 cm^3 concentrated ortho-phosphoric acid was added. A volume of 1 cm^3 indicator and titrate excess dichromate with iron (II) ammonium sulphate solution was added. As the endpoint approached, the solution colour changed to dark violet brown. Iron (II) ammonium sulphate was added drop by drop until the colour changed sharply to green. The determination was repeated with less soil if more than 75% of the dichromate was reduced.

5.3.5 Extractable phosphorus: Bray 1

Since the quality of P extracted from soil is temperature dependent, the laboratory and extracting solution temperature was kept at 20 ± 2ºC. The next 2.5 cm^3 of soil was scooped into a sample cup and 25 cm^3 extraction solution was added to the soil. The mixture was stirred for 10 minutes at 400 rpm, and finally the filtrate was extracted into a clean sample cup, using Whatman no1 filter paper.

5.3.6 Soil Ph

a) pH (KCl)

The pH meter was calibrated at a constant temperature with commercially available standard buffer solutions, and re-calibrated hourly to compensate for drift. A weight of 10 g dried soil (≤ 2 mm) was placed in a glass beaker followed by adding 25 cm^3 KCl solution (1 mol dm^{-3}). The contents were stirred rapidly for 5 seconds with a glass rod, and they were again stirred after 50 minutes and allowed to stand and settle for 10 minutes. pH was determined with a calibrated pH meter with the electrodes positioned in the supernatant, and the results were presented as pH (KCl). The pH (KCl) is not reliable for interpreting the soil's fertility or crop production potential, hence, pH (H_2O) is performed to obtain the Delta pH (pH (KCl) – pH (H_2O).

b) pH (H_2O)

The procedure was the same as pH (KCl), but after placing 10 g of dried soil (≤ 2 mm) in a glass beaker, 25 cm^3 de-ionised water was added. The readings were reported as pH (H_2O).

5.4 Topographical measurements

5.4.1 Bare-ground, fixed rock and stone cover

The total percentages (in categories of 20% intervals) of area that all the three abiotic features covered in a radius of 15 cm around an individual plant were recorded. A numeric code system was used to simplify data collection and statistical analysis (Table 4.1).

Table 4.1 Conversions of percentage cover to numerical codes.

Percentage cover (%)	Numeric code
0	0
1-20	1
21-40	2
41-60	3
61-80	4
81-100	5

5.4.2 Aspect and slope

Aspect and slope were recorded during field survey using a GPS, compass and topographical maps. A numerical code system was also used for various cardinal points and different slope angles.

5.5 Statistical analysis

For statistical analysis the IBM Statistical Package for Social Sciences (SPSS) version 22 software package was used to analyse the population structure and size, flowering, fruiting, fire damage, and abiotic features for individual populations. This was done by; cross-tabulating the desired variables in columns and rows using descriptive statistics, and the tables were exported to Microsoft Office Excel 2007 software from which Histogram graphs were constructed. SPSS was further used to perform the Pearson's correlations test on various data sets. Correlation tests are performed to establish the relationship between two variables (Freedman, 2000). Scatterplot and box-and-whiskers graphs were constructed on correlating variables using SPSS.

In statistics, analysis of variance (ANOVA) is a collection of statistical models, and their associated procedures, in which the observed variance in a particular variable is partitioned into components attributable to different sources of variation. ANOVA provides a statistical test of whether or not the means of several groups are equal, and therefore generalizes the t-test to more than two groups (Corder and Foreman, 2009). A one-way ANOVA test, in combination with Homogeneity of Variances Test was performed on the canopy sizes of all sampled populations in order to ascertain significant differences. The results from the one-way ANOVA test does not indicate which of the groups in a variable and/or variables differs from which, so a follow-up analysis with a Tukey HSD Post Hoc test was performed to counter this deficiency.

6 RESULTS

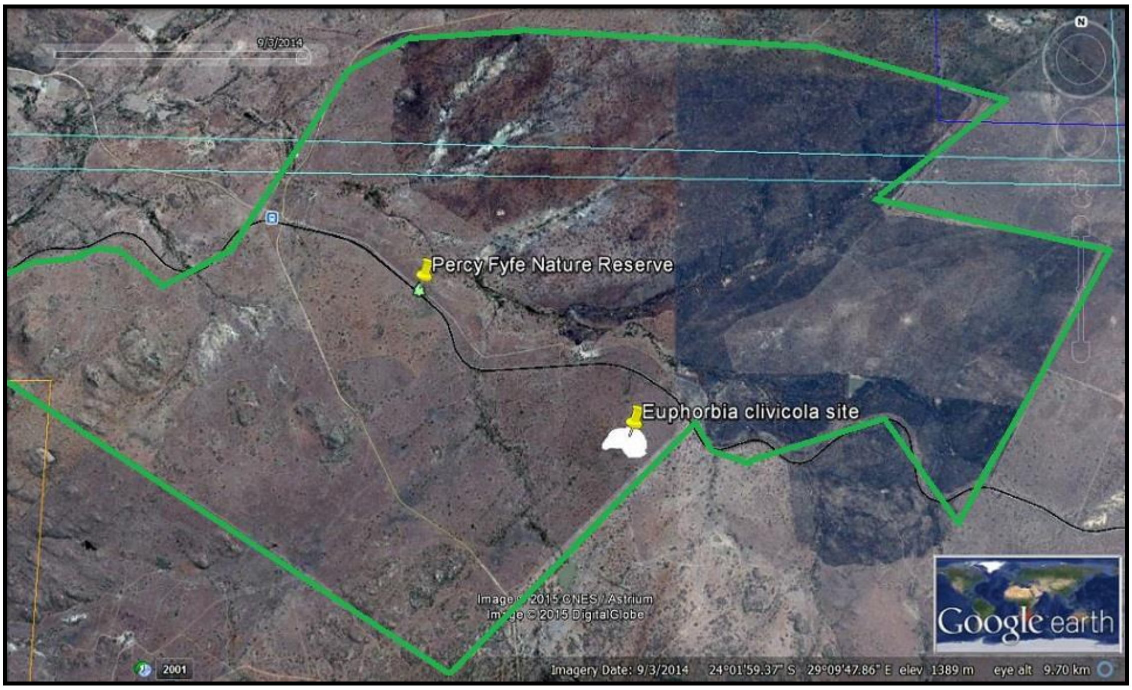
6.1 Population biology

6.1.1 Area of Occupancy

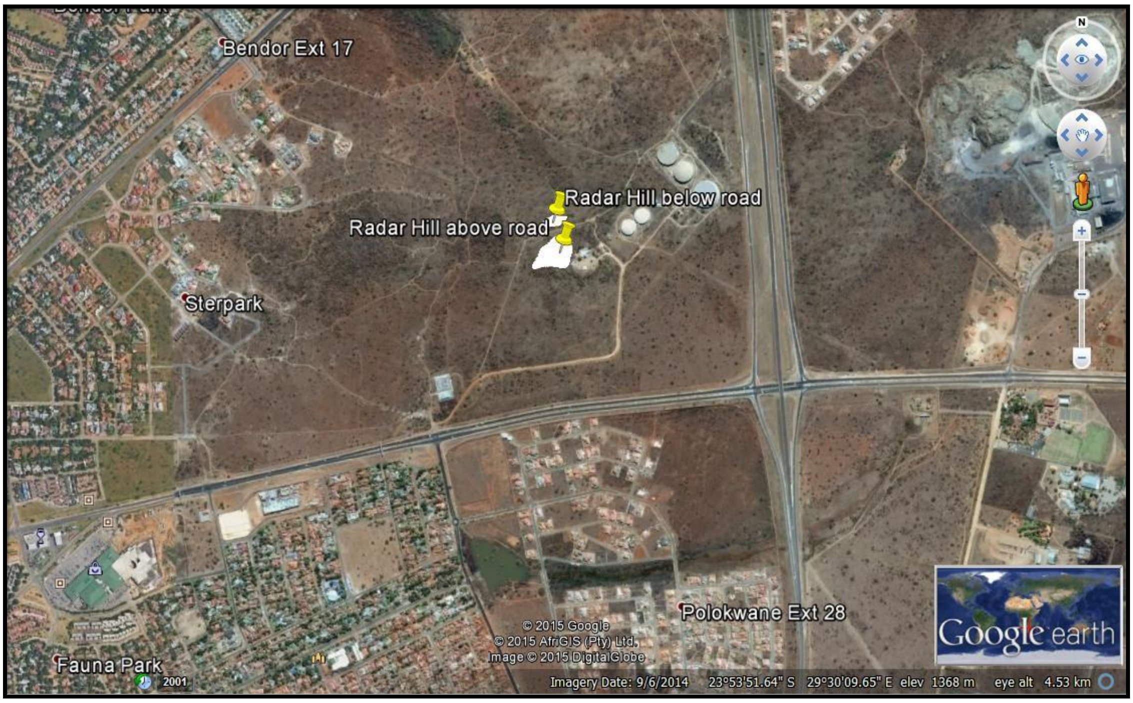
The surface areas occupied by populations of *E. clivicola* were quantified to inform the type of sampling method to be used. The area occupied by populations at; Percy Fyfe Nature Reserve (Figure 4.7a) was 11 428.64 m^2, Radar Hill (Figure 4.7b) was 14 984.71 m^2 and Makgeng below R71 road (Figure 4.7d) was 5 632.21 m^2 (see Table 4.2 for AOO of all populations). The Nearest Individual Sampling Technique (NIST) was used due to the smaller Area of Occupancy on the above mentioned populations.

Larger populations were observed at Dikgale subpopulation A and B (Figure 4.7c) and Makgeng above (Figure 4.7d) R71 road. Areas of occupancy for Dikgale subpopulation A, subpopulation B, and Makgeng above R71 road is 133 595.52 m^2, 112 273.16 m^2 and 65 738.20 m^2, respectively (Table 4.2). The Centre-Quarter Method was used to sample large populations mentioned above.

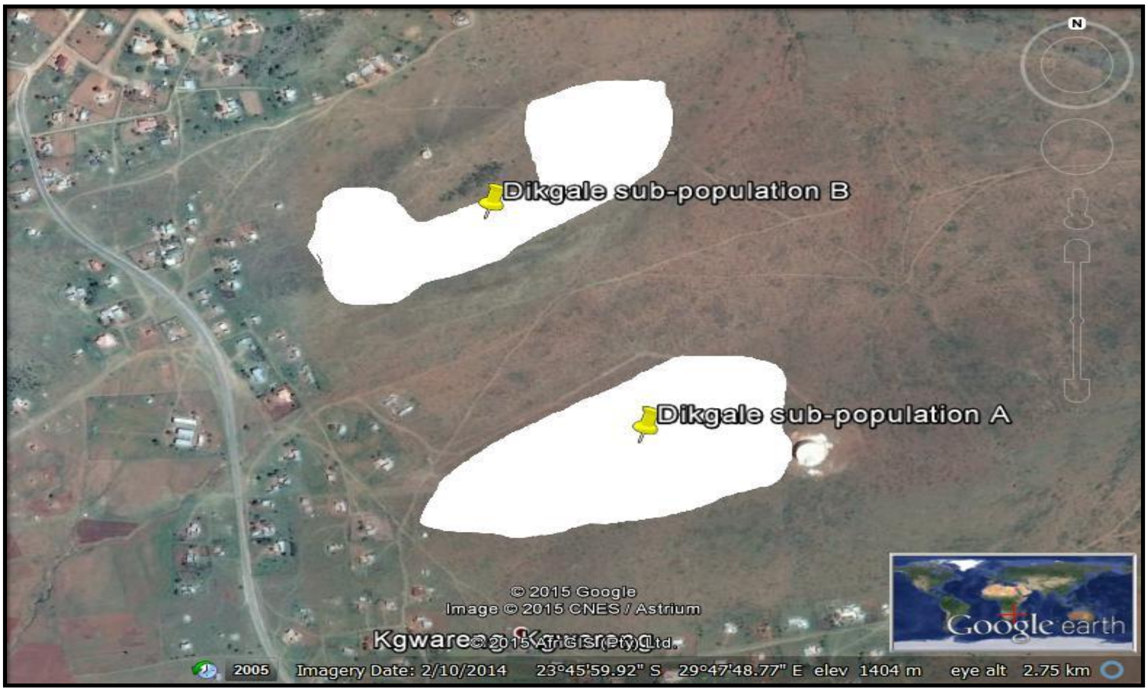
a)


Percy Fyfe Nature Reserve
Euphorbia clivicola site
Image © 2015 CNES / Astrium
Image © 2015 DigitalGlobe
Google earth
2001
Imagery Date: 9/3/2014 24°01'59.37" S 29°09'47.86" E elev 1389 m eye alt 9.70 km

b)


Bendor Ext 17
Radar Hill below road
Radar Hill above road
Sterpark
Polokwane Ext 28
Fauna Park
© 2015 Google
© 2015 AfriGIS (Pty) Ltd.
Image © 2015 DigitalGlobe
Google earth
2001
Imagery Date: 9/6/2014 23°53'51.64" S 29°30'09.65" E elev 1368 m eye alt 4.53 km

c)



d)

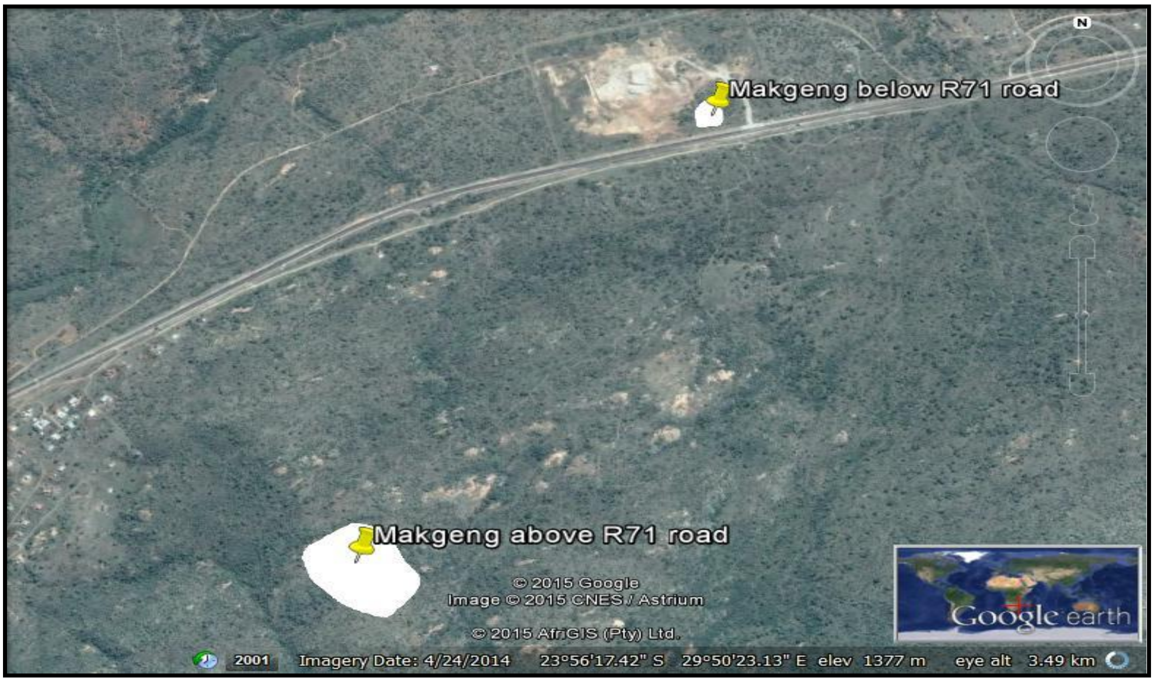


Figure 4.7 Area of occupancy for the four study sites; a) Percy Fyfe Nature Reserve, b) Radar Hill (below and above road), c) Dikgale (subpopulation A and B), and d) Makgeng (below and above the R71 road).

6.1.2 Number of individuals per population

An estimation of the number of individuals in a population was determined for the populations sampled via PCQ. The number of individuals in a particular population was determined by multiplying the area of occupancy with the average density [Density = $1/(2D_m)^2$]. Dikgale subpopulation A and Dikgale subpopulation B comprised of an estimated 37 006 and 30 145 individuals respectively, while Makgeng above R71 road population consisted of an estimated 13 338 individuals (Table 4.2).

Table 4.2 An estimation of areas of occupancy occupied by all known populations of *Euphorbia clivicola*.

Populations	Average density (m^2)	Estimated area of Occupancy (m^2)	Area sampled (%)	Number of individuals
Percy Fyfe Nature Reserve	0.0037	11 429	100	8
Radar Hill above road	0.2357	14 985	100	176
Radar Hill below road	0.0047	5 239	100	11
Dikgale Population A	0.2770	133 596	19	37 006*
Dikgale Population B	0.2685	112 273	25	30 145*
Makgeng below R71 road	0.0631	5 632	100	11
Makgeng above R71 road	0.2029	65 738	38	13 338*

* Estimated number of individuals (refer top for calculations).

6.1.3 Life stages

Juvenile plants were absent in Percy Fyfe Nature Reserve, Radar Hill (below the gravel road), and Makgeng below R71 road (Table 4.2). Seedlings were present in all populations, except Radar Hill (below road) and Makgeng (below R71 road). Percy Fyfe Nature Reserve population was the smallest population with only 8 living and 6 senescent individuals; 50% of adults (7) (Table 4.2) and 7.10% of seedling (1). All populations were dominated by adult plants ranging between 50% (Percy Fyfe Nature Reserve) and 100% (Radar Hill below road and Makgeng below R71 road), while senescent plants were observed in all populations. Percy Fyfe Nature Reserve contained the highest percentage of senescent plants at 42.90 (Figure 4.8).

Table 4.3 Population distribution estimates of all known populations of *Euphorbia clivicola*.

		POPULATION						
	AGE CLASS	PERCY FYFE NR	RADAR HILL ABOVE	RADAR HILL BELOW	DIKGALE POP A	DIKGALE POP B	MAKGENG ABOVE	MAKGENG BELOW
POPULATION SIZES	senescent	6	4	0	3 923	2 894	1 801	0
	adult	7	158	19	28 643	24 056	10 004	19
	juvenile	0	13	0	3 368	2 894	1 027	0
	seedling	1	5	0	1 073	302	507	0
Total		14	180	19	37 006	30 145	13 338	19

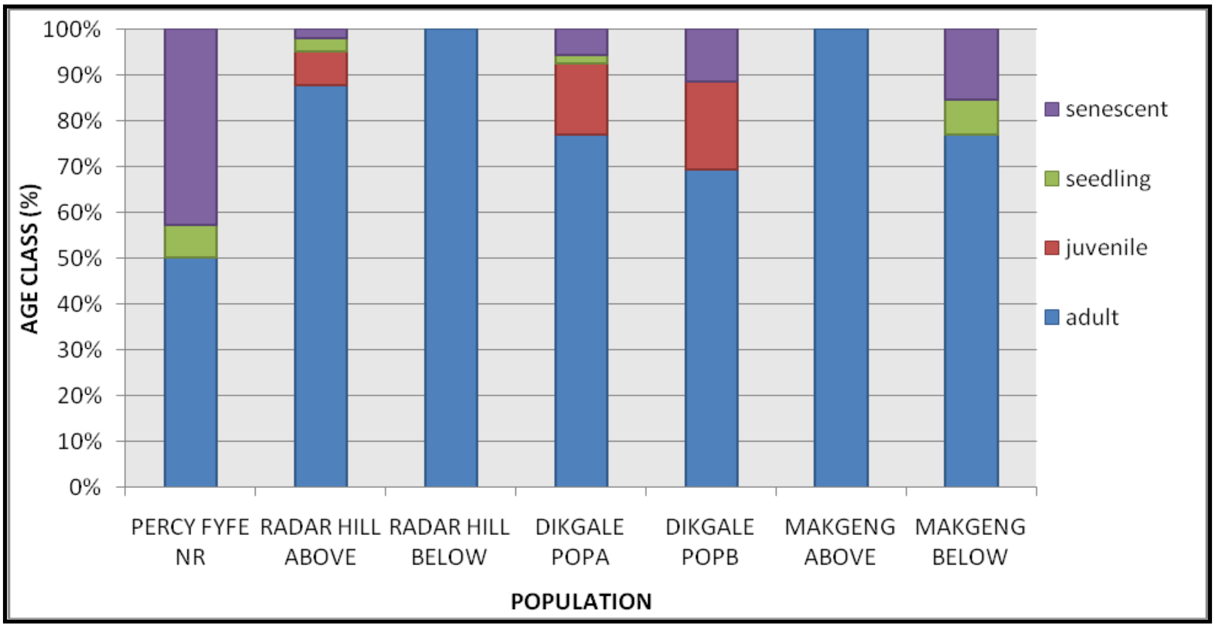


Figure 4.8 A visual representation of age classes of all known populations of *Euphorbia clivicola*.

6.1.4 Canopy size structure

The canopy sizes of *E. clivicola* plants ranged from an area of 0.79 cm^2 to an area of > 2000 cm^2 (Figure 4.9). Canopy areas per population were compared using a One-Way ANOVA Test and a significant difference was observed ($P < 0.01$). A Tukey Post Hoc Test was used to determine where this difference layed. Variations from very weak to weak positive correlations were observed to range between r = 0.001 and 0.043. The Percy Fyfe Nature Reserve population was the only one that had no plants with a canopy area of >2000 cm^2 (Figure 4.9), and the mean canopy size was the smallest at 2.64 cm^2. The Radar Hill below the road and Makgeng below the R71 road subpopulations had the highest mean canopy sizes of 8.27 and 7.58 cm^2, respectively. The remaining populations' mean canopy size ranged between 4.38 and 8.23 cm^2 (Figure 4.10).

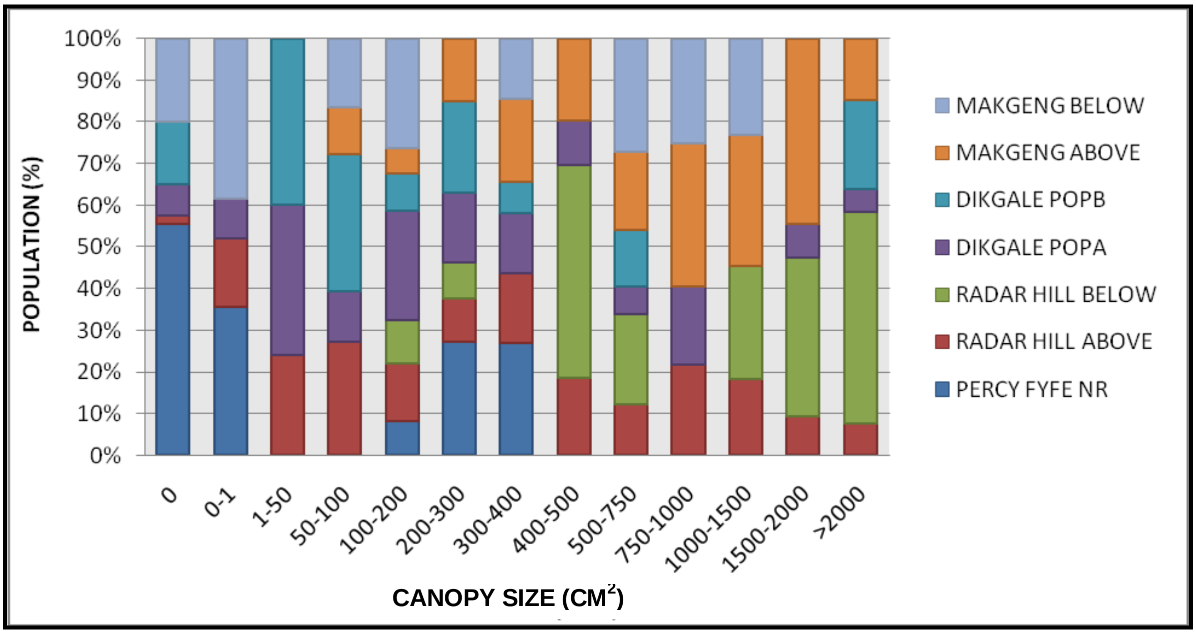


Figure 4.9 The size structure (canopy area) of all known populations of *Euphorbia clivicola*.

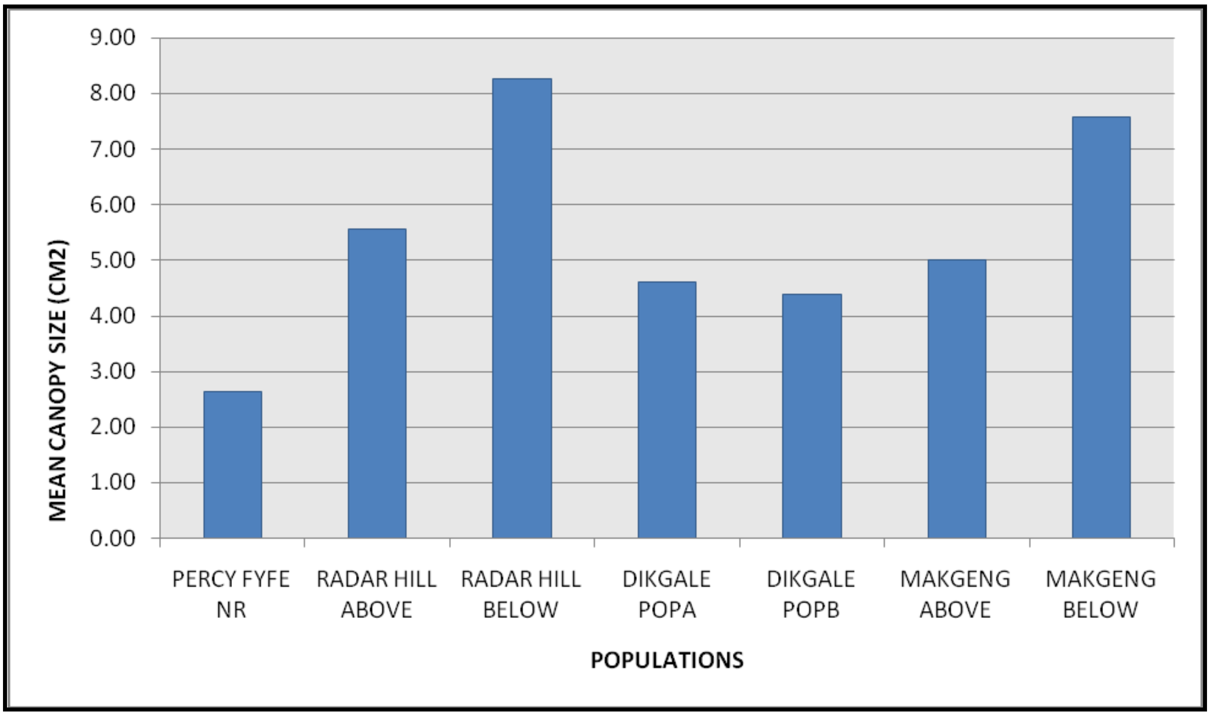


Figure 4.10 The means for the total canopy sizes of all known populations of *Euphorbia clivicola*.

6.1.5 Recruitment capacity

The recruitment capacity survey was conducted from September to October of 2013. Most of the populations of *E. clivicola* differed significantly regarding the number of plants carrying flowers with weak to moderate correlations ($P < 0.05$, r = 0.30—0.51). The Radar Hill below road subpopulation had the second highest percentage of plants with flowers (45.5%) (Figure 4.11), but had 0% fruits (Figure 4.12). The Makgeng below the R71 road subpopulation had the highest percentage of plants with flowers (74.2%) (Figure 4.11), and equally the highest percentage of plants with fruits (30.8%) (Figure 4.12).

Euphorbia clivicola plants with a canopy area less than 50 cm^2 were non-flowering (Figure 4.13), and subsequently did not produce fruits. Canopy sizes ranging between 200 cm^2 to 1500 cm^2 were observed to flower (6.7—41.7%) (Figure 4.13) and fruit (2.9-60%) (Figure 4.14) the most. Plants with a canopy area greater than 2000 cm^2 possessed > 100 flowers (83.3%) per plant, while plants with a canopy area of between 750 and 1000 cm^2 produced the highest number of fruits >100 per plant (30%) (Figure 4.14).

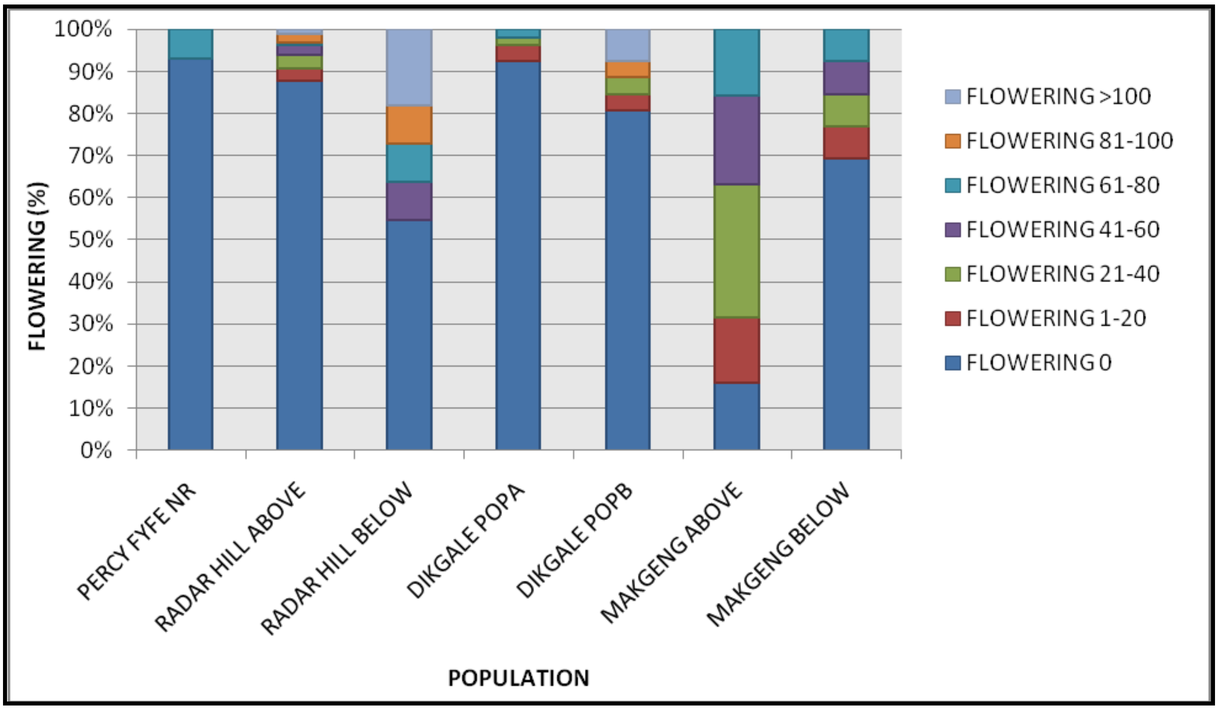


Figure 4.11 The percentage of flowering and non-flowering plants in each population of *Euphorbia clivicola*.

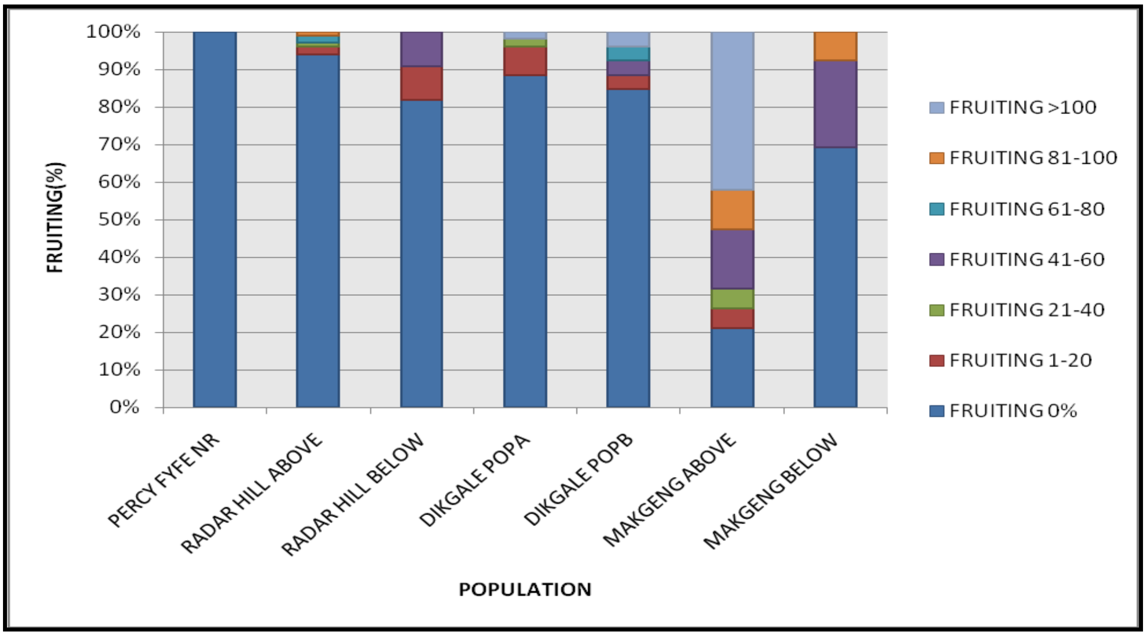


Figure 4.12 The percentage of fruiting and non-fruiting plants of each population of *Euphorbia clivicola*.

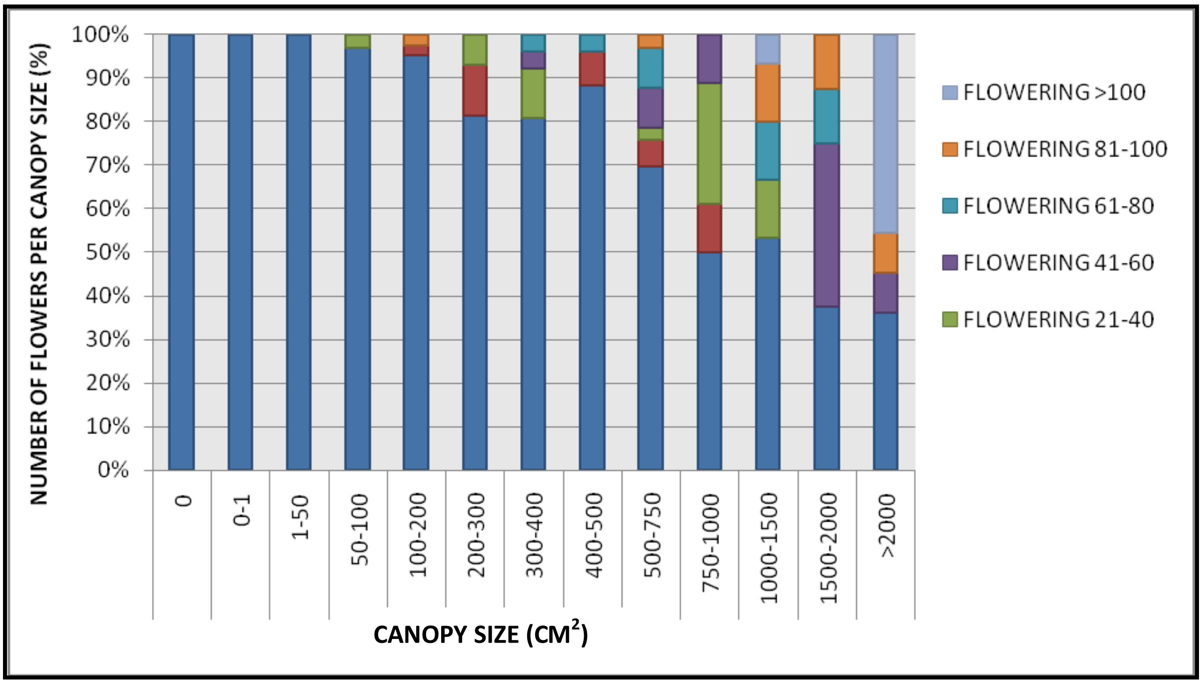


Figure 4.13 The percentage of flowering and non-flowering plants in each size class from all known populations of *Euphorbia clivicola*.

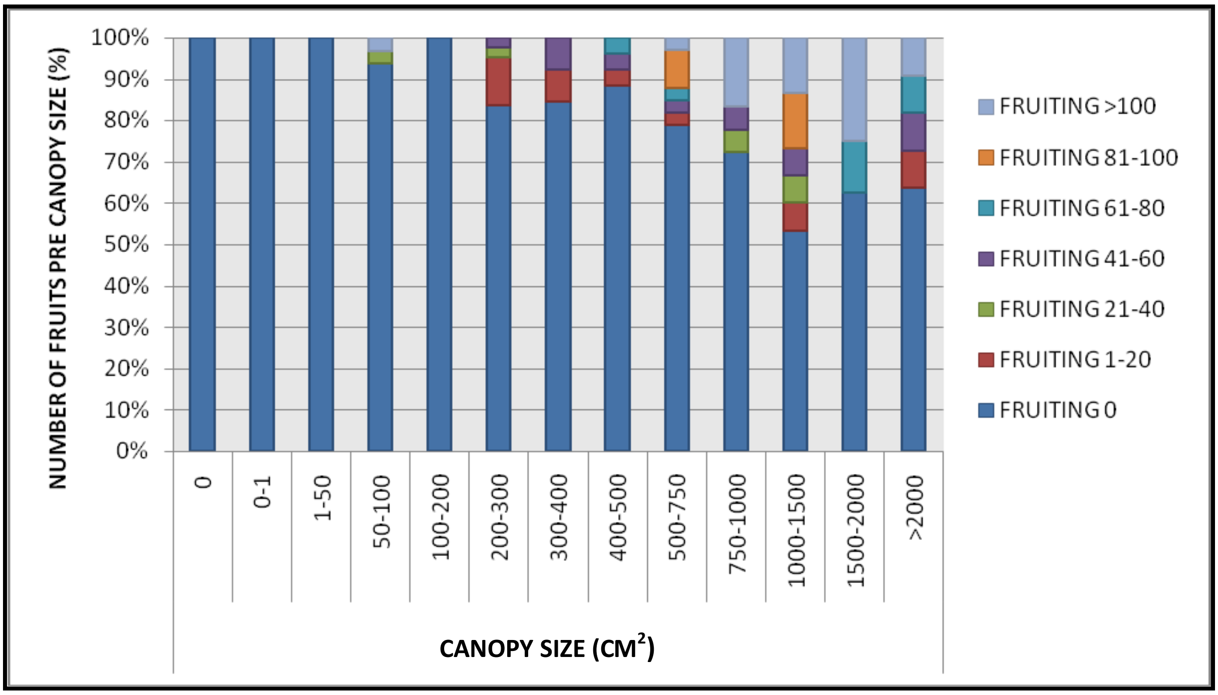


Figure 4.14 The percentage of fruiting and non-fruiting plants in each size class of all populations of *Euphorbia clivicola*.

6.2 Abiotic factors

6.2.1 Fire

Only two subpopulations of Radar Hill above road and Makgeng above R71 road were affected by fire during the course of the study. Fires occurred during winter of 2013. Forty-seven percent of the plants at Radar Hill above road subpopulation sustained different levels of damage caused by fire, and 92% of the Makgeng above R71 road plants were also damaged by fire. From the 47% of damaged plants at the Radar Hill subpopulation, 15% showed less than 20% damage, and 20% of the plants had 21—40% damages (wilted). A minute number (6%) of the Radar Hill population sustained intense fire damage, ranging from 61 to 100%. The fire in the above R71 road (other affected) population of Makgeng showed more intense damage, as 54% of the plants affected by fire sustained 61—80% damage, while 8% suffered 81—100% fire damage (Figure 4.15).

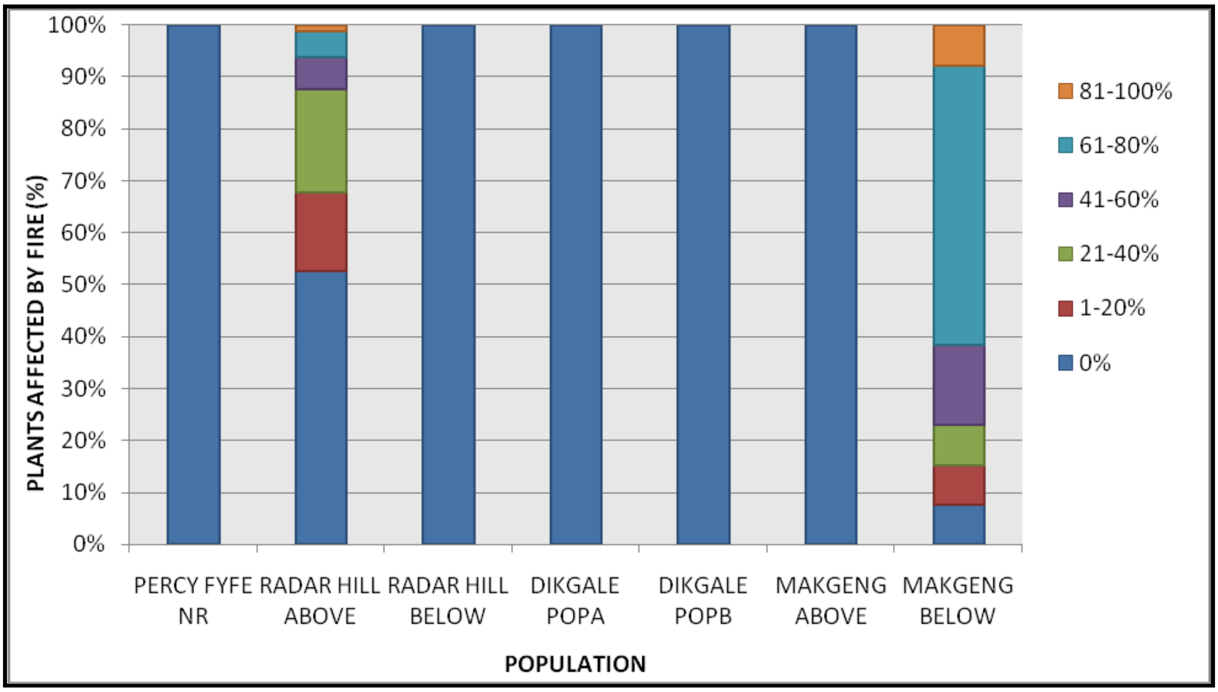


Figure 4.15 Populations of *Euphorbia clivicola* that were affected by fire and the percentage of damage the fire caused to the plants.

Seedlings and juvenile plants were not affected by fire. Fire damage of 81—100% were mostly observed on plants with a canopy area > 1 cm^2 and < 1500 cm^2. The

Radar Hill above road subpopulation had only 12% of plants reflecting damage of > 41%, while Makgeng above R71 road contained 78% of plants with > 41% fire damage (Figure 4.16).

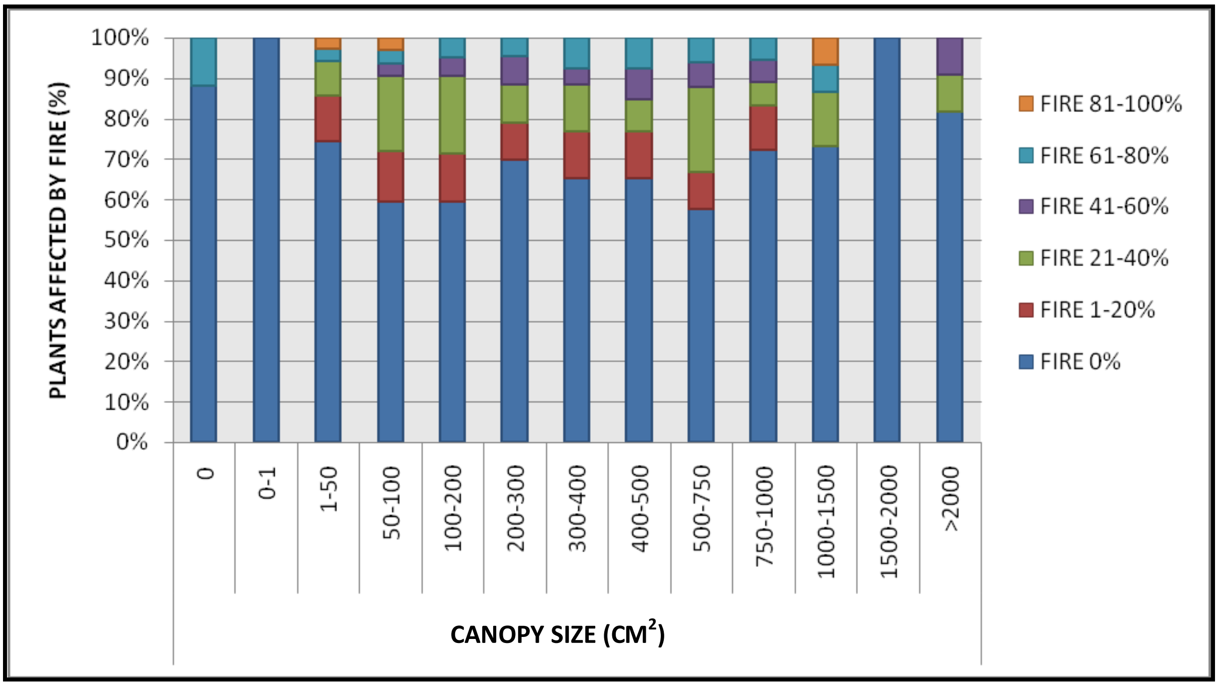


Figure 4.16 Percentage damage caused by fire across a variety of canopy sizes of *Euphorbia clivicola* at the Radar Hill above road and Makgeng above R71 road populations.

The responses of *E. clivicola* after the fire were monitored at the Radar Hill population. New growth was observed on all plants that were affected by fire. A strong positive correlation between damage and regrowth was observed ($P < 0.01$ and r = 0.89). Plant damage was observed to be directly proportional to resprouting; because 51—60% of damaged plants produced 91—100% new growth, while plants which sustained 1—5% damage were observed to show less than 10% regrowth (Figure 4.17).

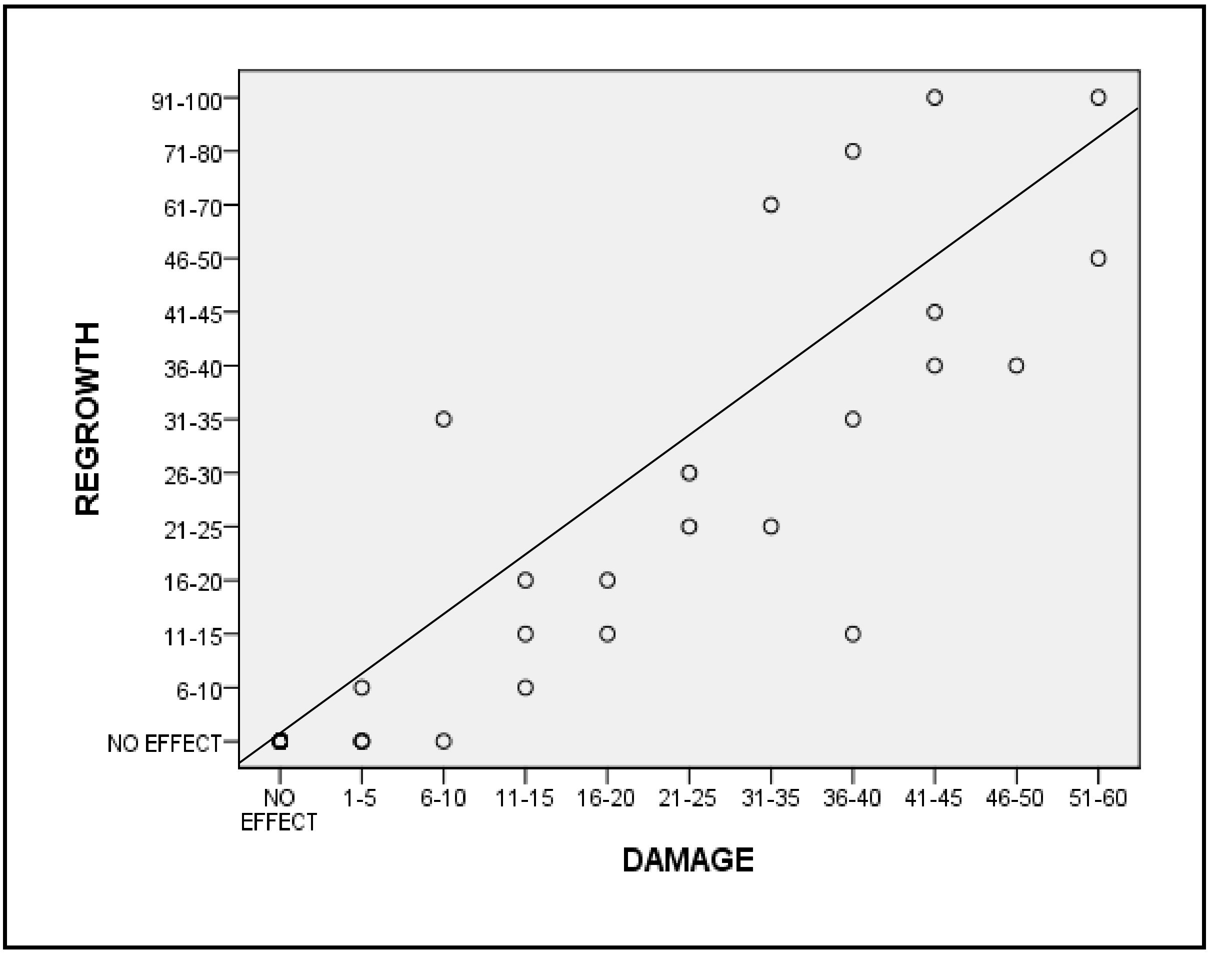


Figure 4.17 The percentage of *Euphorbia clivicola* plants showing directly proportional regrowth response after experiencing various fire damage.

6.2.2 Abiotic features cover

a) Bare ground cover

The Dikgale subpopulation A had 77%, Dikgale subpopulation B (69.2%), Makgeng above R71 road (61.6%), and Radar Hill subpopulations (38.9%) of plants occupying habitats with a bare ground cover of less than 40%. A 92.9% total of plants from Percy Fyfe Nature Reserve population occupied an area that had bare ground cover ranging between 61 and 100% (Figure 4.18). A Post Hoc Test (Tukey HSD) revealed weak correlations ($P < 0.05$ and r = 0.000—0.043) between all populations, except for Dikgale subpopulation A and B, and Radar Hill above and below the road ($P > 0.05$).

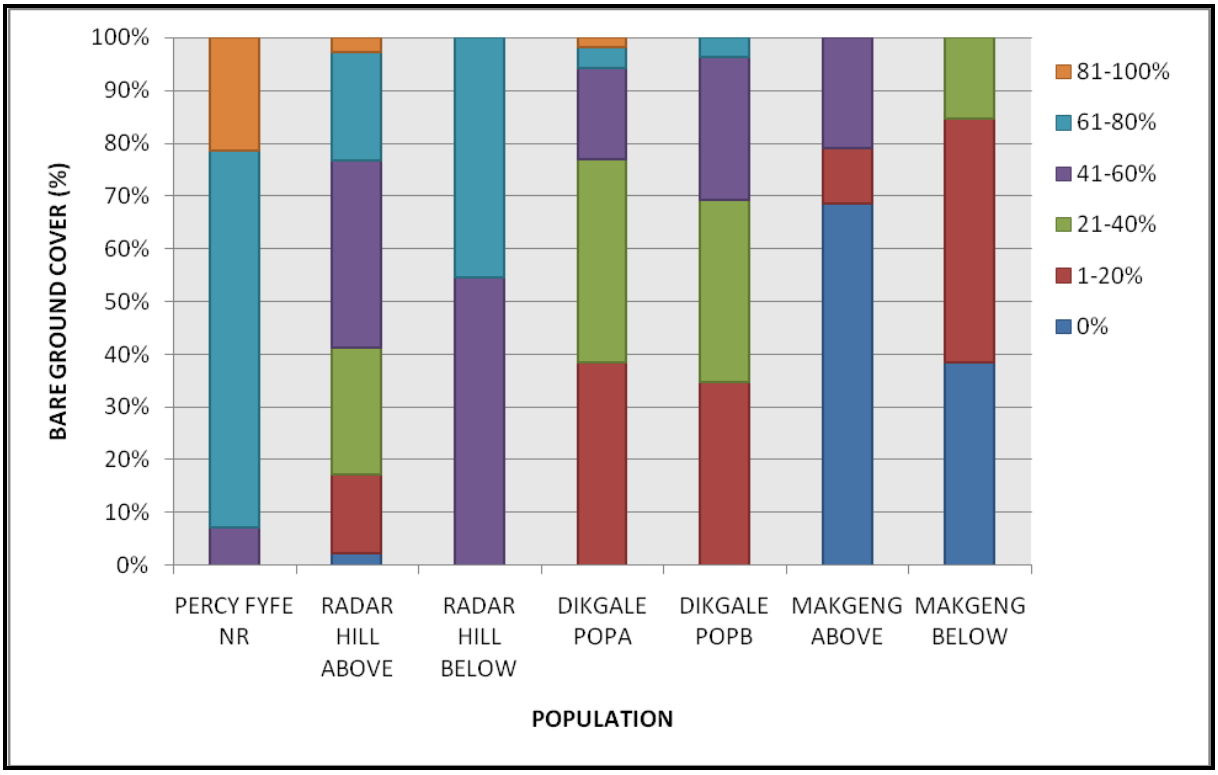


Figure 4.18 Total percentage of bare ground covered in a radius of 15 cm around individual plants of *Euphorbia clivicola* from all known populations.

b) Fixed rock cover

It is evident that *E. clivicola* prefers rocky habitats (Figure 4.19). An average of 30% of plants in all known populations occupied habitats with a fixed rock cover of 41—60%, while 29% of plants inhibited areas with 21—40% rock cover. Only 10.6% of the plants across all sampled populations occupied habitats with no rock cover (Figure 4.20).

Figure 4.19 Example of the rocky habitat *Euphorbia clivicola* typically occurs.

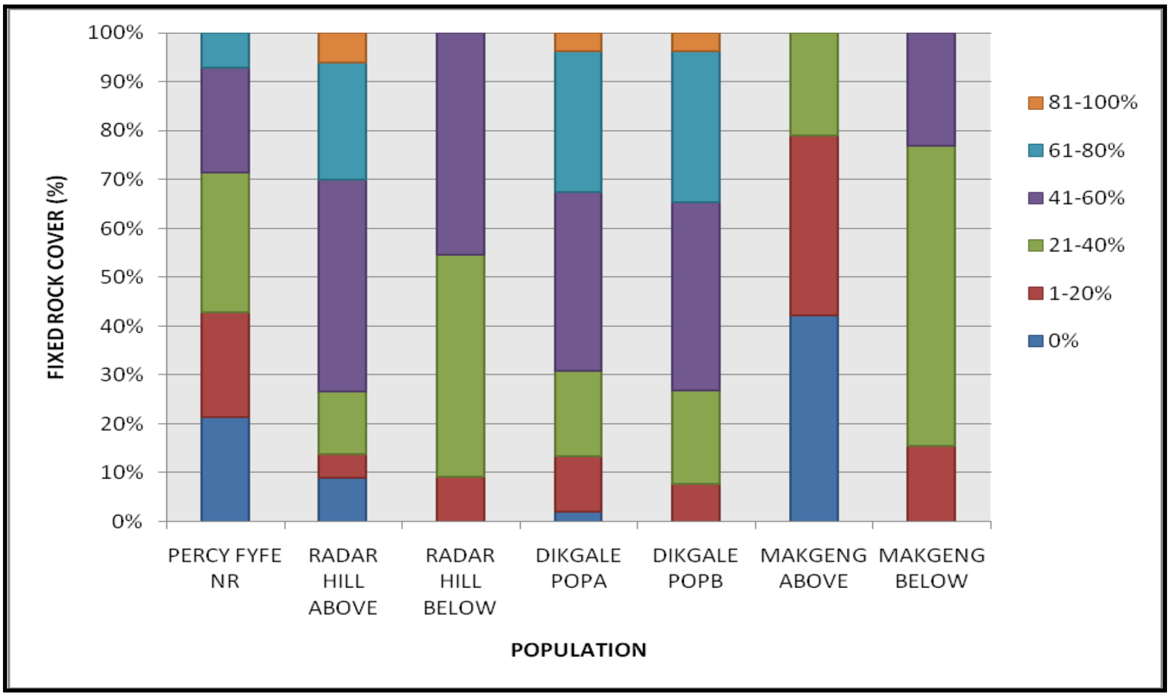


Figure 4.20 Total percentage of fixed rock cover in a radius of 15 cm around individual plants of *Euphorbia clivicola* from all known populations.

c) Stone cover

Euphorbia clivicola species occur in rocky and stony terrains in all known populations. Stone cover per population were correlated (Pearson's Correlation, $P < 0.01$ and r = 0.29), with 27.7% of all populations preferring habitats with stone cover of 21 to 40%, while 36.3% of plants occupy sites with 41—to 60% stone cover (Figure 4.21). Fixed rock cover and stone cover also appeared to be negatively correlated ($P < 0.01$ and r = - 0.16); because as stone cover increases from 20—100%, fixed rock cover decreases from 80 to 0% (Figure 4.22).

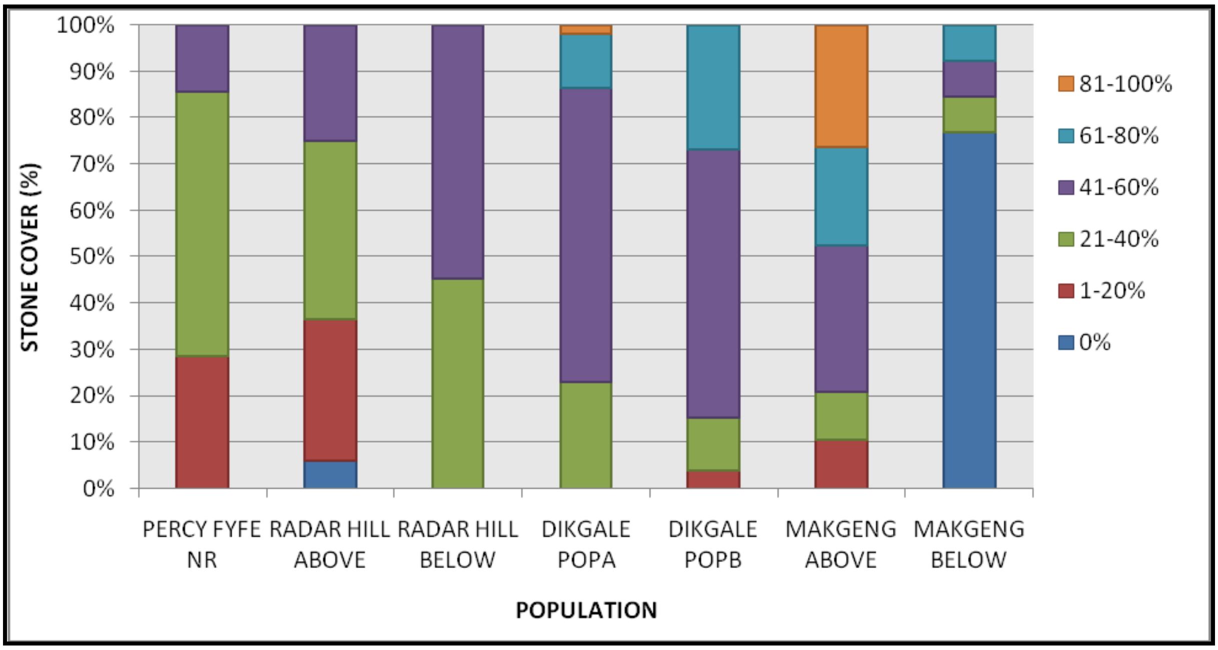


Figure 4.21 Total percentage of stone cover in a radius of 15 cm around individual plants of *Euphorbia clivicola* from all known populations.

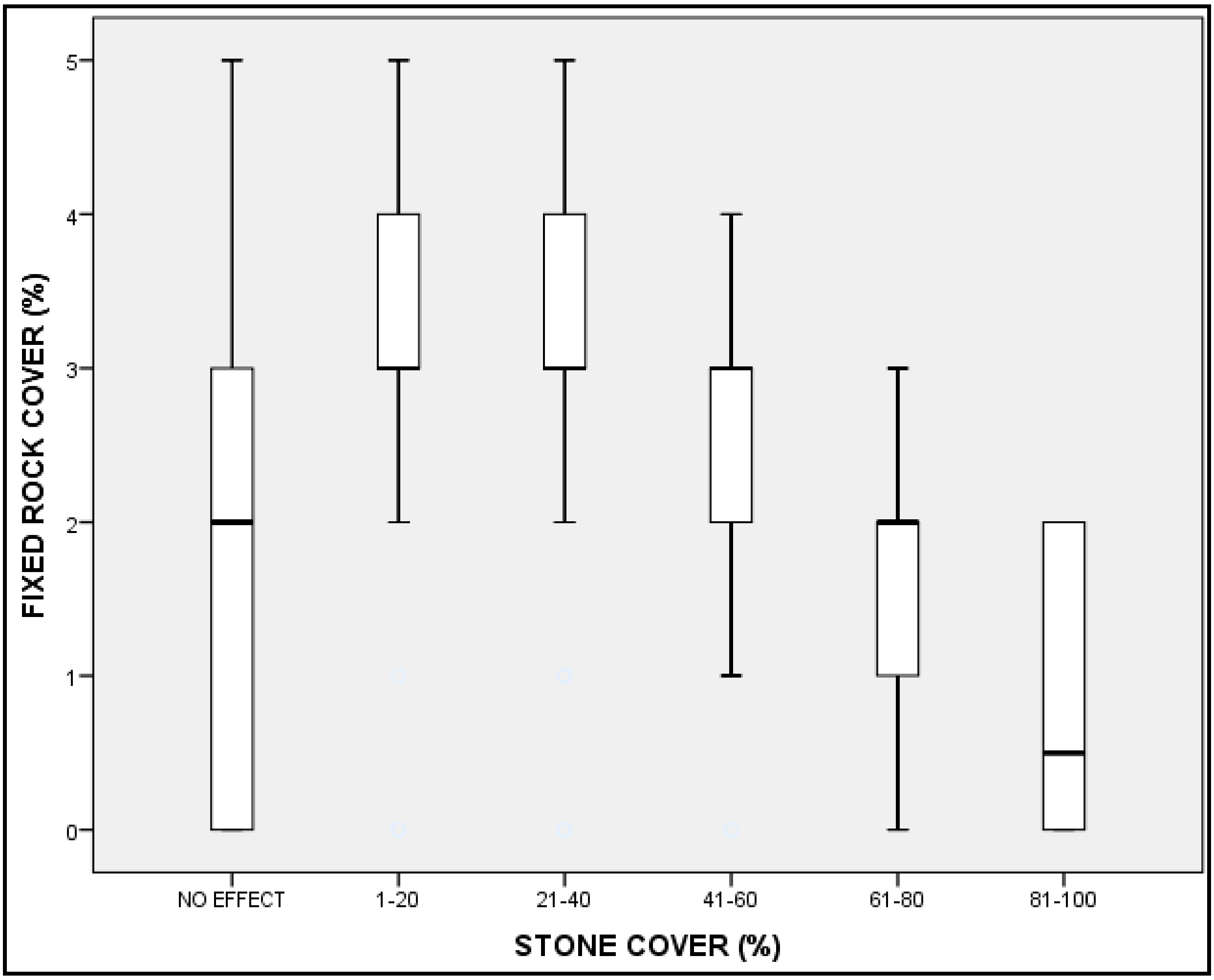


Figure 4.22 The relationship between fixed rock cover and stone cover across all known populations of *Euphorbia clivicola*. Fixed rock cover: 1 = 1—20%, 2 = 21—40%, 3 = 41—60%, 4 = 61—80% and 5 = 81—100%.

6.2.3 Aspect and slope

Although four (Percy Fyfe Nature Reserve, Radar Hill below the road, Makgeng below and above) out of all known populations of *E. clivicola* and *Euphorbia* (*incertae sedis*) occurred on the north-facing (N) slopes (Figure 4.23), no significant correlation (Pearson's Correlation $P > 0.01$) was observed between all populations and aspect.

The Radar Hill above road subpopulation was predominantly (85.6%) found on the north-western (NW) aspect. Dikgale subpopulation A was the only one with *E. clivicola* (26.9%) plants growing on the south-western (SW) aspect and, 28.8% and 38.5% inhibited the north-western and north-eastern (NE) aspects, respectively. Dikgale subpopulation B consisted of 23.1%, 15.4%, and 61.5% on the N, NW, and NE, respectively (Figure 4.23).

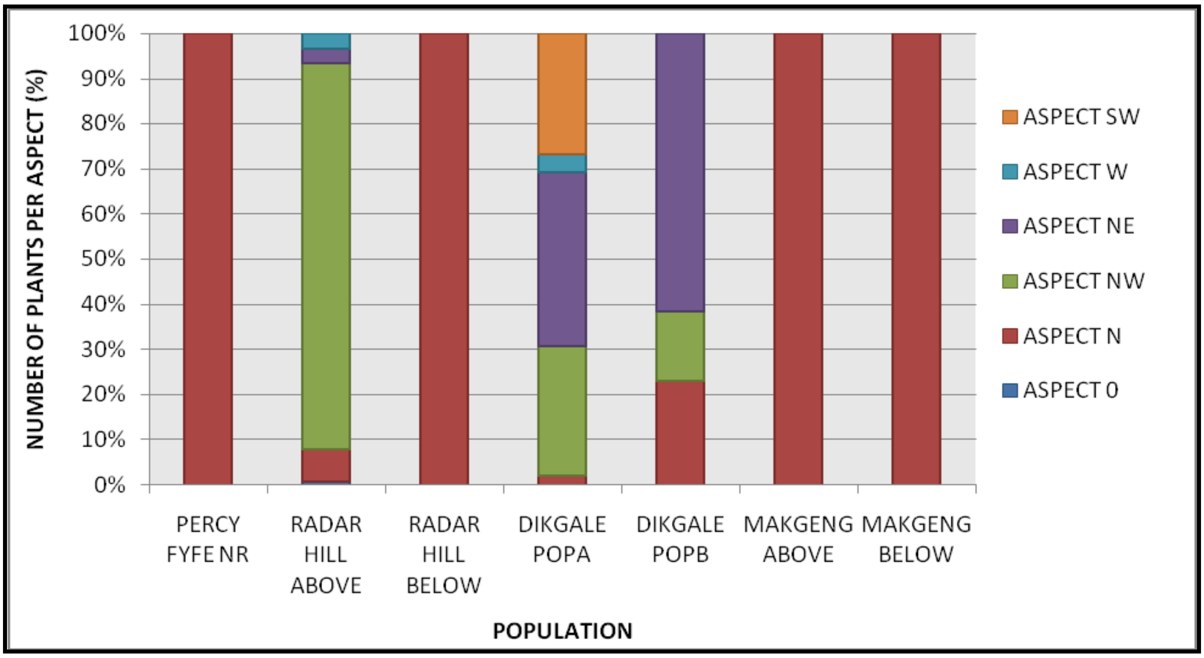


Figure 4.23 Total population percentage of *Euphorbia clivicola* plants per aspect.

There was a clear negative correlation between the slope degree within populations ($P < 0.01$ and r = - 0.32). A greater proportion (70.9%) of *E. clivicola* prefer to grow on slopes ranging from less than 5° up to 11°. All plants at the Radar Hill below the road population occured on slopes with an incline of 0—5° (Figure 4.24).

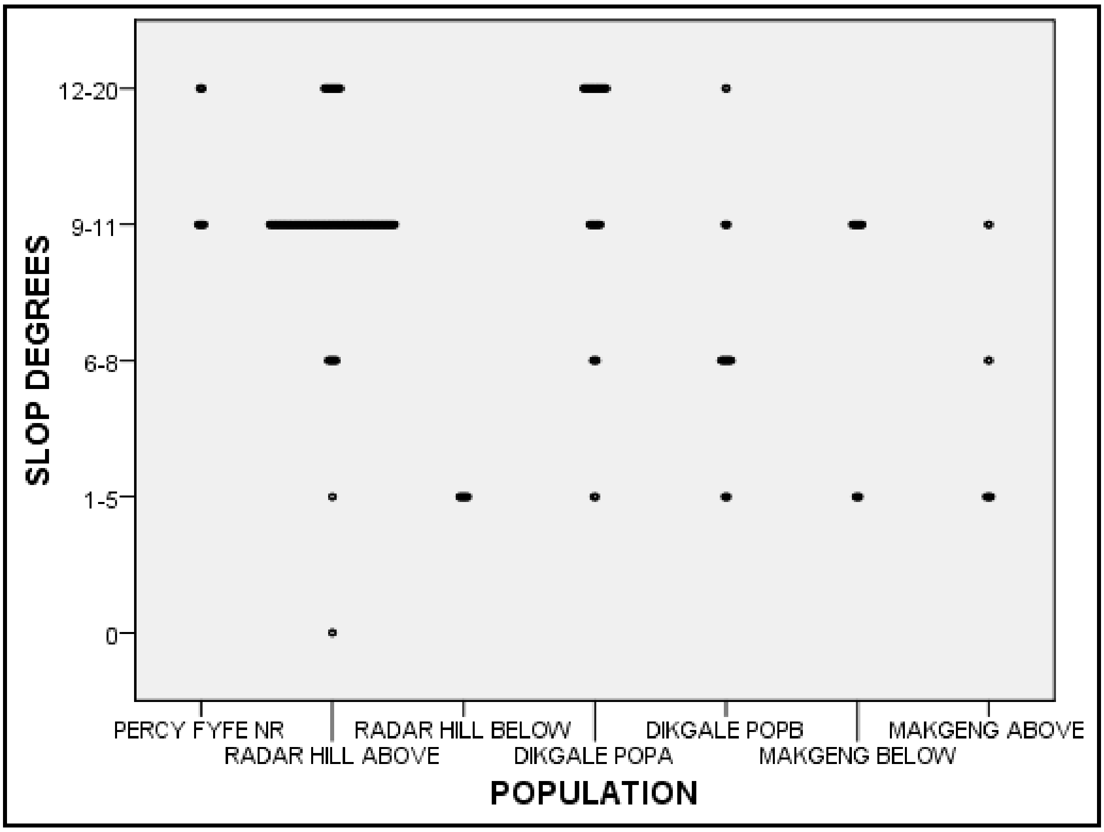
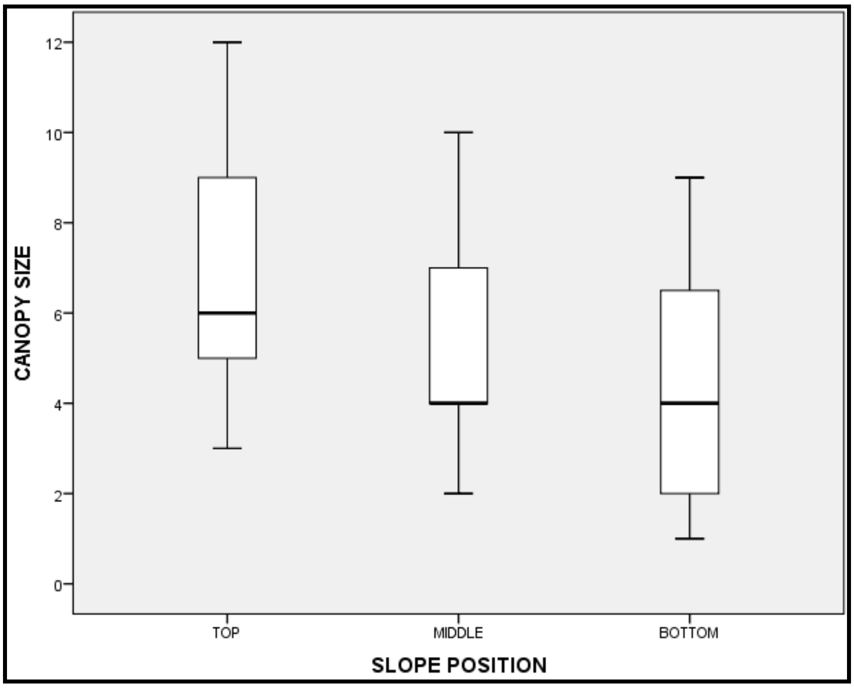


Figure 4.24 A summary of the total population percentage of *Euphorbia clivicola* plants per slope degree. The wider width of symbols on the plot represents higher number of sampled plants in that cartegory.

Slope position at the Radar Hill, Dikgale, and Makgeng above R71 road subpopulations were observed to be negatively correlated with the canopy sizes of *E. clivicola*; Pearson's Correlation was $P < 0.01$ and r = - 0.42. Also at the above mentioned populations, density was positively correlated to slope position; Pearson's Correlation $P < 0.01$ and r = 0.37. The canopy of *E. clivicola* plants gradually decreased in size (Figure 4.25a), and the distance between individual plants increased along the slope from top to bottom (Figure 4.25b). It should be noted that at Radar Hill above the road on the top of the hill (flat surface), a small number of *E. clivicola* plants occurred. This was the only flat surface (< 10%) area were *E. clivicola* plants occurred across all populations.

a)



b)

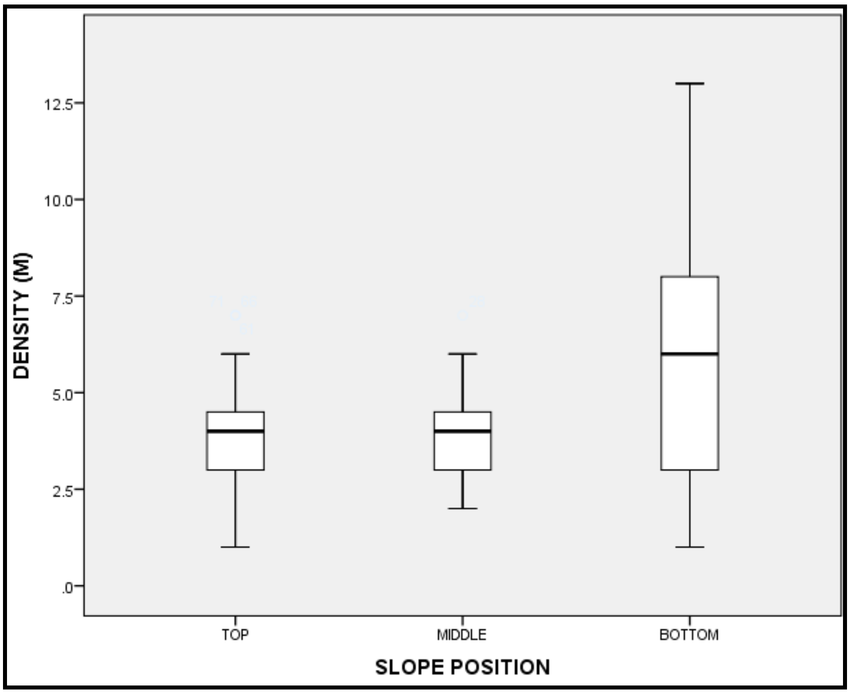


Figure 4.25 Slope position of *Euphorbia clivicola* from Radar Hill, Dikgale and Makgeng above R71 road; a) different slope positions versus the canopy sizes (2 = 1—50, 4 = 100—200, 6 = 300—400, 8 = 500—750, 10 = 1000—1500, and 12 = > 2000 cm) of the plant, b) different slope positions versus density.

6.2.4 Soil

The soils from all known *E. clivicola* populations were found to be acidic. Soils from the Dikgale population contained more silt and clay. Soil texture at all sampled populations were gravel, dominated by sand. Organic matter was high at Makgeng and Radar Hill population. Sodium (Na) was found to be high at Percy Fyfe Nature Reserve and Dikgale (Table 4.4).

Table 4.4 Habitat soil characteristics of all *Euphorbia clivicola* populations.

Soil characteristics	**Percy Fyfe Nature Reserve**	**Radar Hill**	**Dikgale**	**Makgeng**
pH (KCl)	4.54 ± 1.01	4.68 ± 0.81	4.73 ± 1.13	4.66 ± 0.41
pH (H_2O)	6.37 ± 0.45	6.49 ± 0.21	6.51 ± 0.87	6.41 ± 0.66
Gravel (%) (> 2 mm)	57.61 ± 12.94	46.33 ± 12.78	61.02 ± 7.5	41.46 ± 4.18
Sand (%) (50 - 2000 µm)	62.43 ± 2.89	66.78 ± 2.78	54.49 ± 1.63	69.31 ± 3.21
Silt (%)	18.39 ± 1.27	21.09 ± 3.81	24.12 ± 7.51	23.43 ± 4.33
Clay (%)	21.15 ± 3.74	20.46 ± 3.42	23.87 ± 4.01	17.69 ± 2.98
Organic matter (mg/g)	13.68 ± 4.22	37.74 ± 8.16	28.96 ± 5.01	41.58 ± 4.38
K (mg/g)	13.17 ± 1.29	8.41 ± 0.36	5.88 ± 0.25	38.76 ± 3.89
Na (mg/g)	71.02 ± 4.36	26.41 ± 3.45	63.53 ± 5.16	12.10 ± 1.03
Ca (mg/g)	4.25 ± 0.48	21.74 ± 3.23	47.13 ± 6.78	14.17 ± 2.93
Mg (mg/g)	0.91 ± 0.18	27.23 ± 3.90	11.76 ± 1.74	3.21 ± 4.27
P (mg/g)	0.32 ± 0.06	1.21 ± 0.21	1.93 ± 0.93	0.67 0.08

7 DISCUSSION

7.1 Population biology

7.1.1 Population size

Assessment of the spatial distribution of rare species is one of the main questions to be solved for conservation planning and management. Distribution data is crucial for measuring the size of species' geographic ranges by using Extent of Occurrence (EOO) and Area of Occupancy (AOO), which are globally accepted as surrogates for extinction risk under Red List Criteria (Jimẻnez-Alfaro *et al*., 2012). All known populations of *Euphorbia clivicola* occur on an area with an estimated EOO of 669.3 km^2. Both Percy Fyfe Nature Reserve and Radar Hill populations have dramatically declined in size over the past 24 years. This section partially (population sizes) answers the first research question (Section 3.a) of this Chapter.

a) Percy Fyfe Nature Reserve population

The Percy Fyfe Nature Reserve population has an AOO of 11 428.64 m^2. The population size declined from 1500 individuals in 1986 (Raal, 1986) to 165 individuals in 1997 (Pfab, 1997) and 8 individuals in 2014. Hence, 95% of the population has vanished between 1986 and 2014. This decline could have been due to the exclusion of herbivores in the camp where *E. clivicola* occurs. The elimination of herbivores promoted the growth of a thick sward of grass (graminoids); as such, competition was tilted in favour of the graminoids.

Pfab (1997) recommended that the wild herbivores be removed from this camp as they were threatening the survival of *E. clivicola*, and a fire regime of burning every three years be adopted. From our results and observations the herbivory factor is still in play (Chapter 5), which can only mean that the mountain reedbuck (Pfab, 1997) is not the only herbivore responsible for damage caused to *E. clivicola*. The effects of fire on high biomass production has been advocated by Brys *et al*. (2005), where high rates of biomass production and litter accumulation led to shorter time intervals between subsequent fires and a higher fire severity. This might result in a higher number of incinerated plants, hence the higher number of senescent plants in this population.

b) Radar Hill population

The Radar Hill population declined from 3000 plants in 1986 (Raal, 1986) to 382 individuals (Pfab, 1997) and 176 plants in 2014. 51% of the Radar Hill population have vanished between 1997 and 2014. This drastic decline is can be attributed mostly to development occurring at the peri-urban area where *E. clivicola* occurs. The gravel road developed for cycling fragmented the population and inhibited the secondary dispersal of propagules downhill (Pfab, 1997). The subpopulation below the road had less pollination than the subpopulation above the road, as such, flowers could not be converted to fruit, and recruitment was compromised in the former subpopulation. This could be the result of increased human interaction with the habitat, as such, disturbing the pollinators. This phenomenon has been attributed to the disturbance of the dispersal pattern of *E. clivicola* down the slope by the scrap road, and consequently the subpopulation faces extinction if provisions to connect the two subpopulations are not initiated.

c) Dikgale populations

The populations are widely distributed along the EOO; the two Dikgale subpopulations cover a greater AOO, subpopulation A occupies an AOO of 133 595.52 m^2, while subpopulation B covers 112 273.16 m^2. Dikgale subpopulation A and B have the highest number of individuals; subpopulation A comprised of an estimated 33 084 individuals, while the subpopulation B consisted of approximately 27 254 plants (Chapter 1, Section 3.c). The higher plant density in the two Dikgale subpopulations is associated with the larger AOO, the unperturbed state of the habitat, and other biotic factors (e.g. grazing) that will be presented in Chapter 5.

Reyers (2004) argued that communities or classes that are already transformed should receive high priority over untransformed areas because the latter are usually in-accessible or unsuitable and thus out of harm's way.

The *E. clivicola* habitat in Dikgale is primarily used for grazing domestic animals belonging to the people of Kgwareng village. Due to the higher elevation of the hills compared to the plains in this area, they are also used for construction of water reservoirs (Potgieter, unpublished data). The transformation of the Dikgale subpopulations and the high number of *E. clivicola* plants at the area warrant maximum habitat protection as argued by Reyers (2004).

d) Makgeng population

The Makgeng subpopulations (below and above R71 road) possesses a combined estimate of 11 557 individuals. The subpopulation below R71 road has 19 adults, similar to the Radar Hill below road subpopulation. The two subpopulations mentioned above have the following in common: a) Anthropogenic fragmentation (roads), b) Do not experience fire, c) Greater densities, and d) Little to no recruitment. Anthropogenic activities and the effects of fragmentation are clearly displayed to be detrimental to these two subpopulations.

The estimated number of individuals in the two Dikgale and the Makgeng above R71 road subpopulations positively correlates with their respective AOO, as such; AOO is directly proportional to the number of individuals (abundance) in the *E. clivicola* populations (Chapter 1, Section 3.c). Lawton (1995) has extensively elaborated on the positive correlation between range and abundance of species. In this regard Lawton (1995) noted that fragmentation reduces the AOO, and ultimately the number of individuals will also be reduced. Percy Fyfe Nature Reserve and the other two fragmented populations; Radar Hill below the road and Makgeng below R71 road had low densities, and smaller AOO (5 239.1—32 592.1 m^2).

7.1.2 Life stages

This section explains all known populations' structure of *E. clivicola* and addresses the second part of the first research question (Section 3.a) of this chapter. All sampled populations of *E. clivicola* were greatly dominated by adult plants; with Radar Hill below the road and Makgeng below R71 road composed entirely of adult plants. The inability of the latter two subpopulations to recruit seedlings will definitely lead to extinction. Recruitment must be facilitated (Chapter 6) in the above-mentioned subpopulations to enable them to be sustainable.

For all fragmented or divided populations (Radar Hill and Makgeng populations) the subpopulations located on the down slope (bottom of the slope) have no seedlings or juveniles, as such, it is foreseen that the populations will progressively decline. According to Liao *et al*. (2013), the three components that are triggered by fragmentation, are increased border density, loss of habitat connectivity and reduction of fragmented size are in play at these two subpopulations. The AOO of the two bottom slope fragmented subpopulations are the smallest at < 5 700 m^2 and the density ranges between 0.005 and 0.063 m^2. Reduction of the AOO over time and increasing densities between individual plants will ultimately lead to the extinction of *E. clivicola.* Percy Fyfe Nature Reserve population is also facing the same fate as the two preceding subpopulations, yet it is not fragmented and it is protected. The low density observed in these populations hinders reproduction which will be discussed later in this chapter.

The Makgeng above R71 road population contains individuals from all life stages, but there are more senescent plants than a combination of juveniles and seedlings. Thus 15% of senescent plants are not replaced during each growth season. This phenomenon is attributed to untimed fires and intense grazing experienced by this subpopulation. In the long term this phenomenon will result in the population declining gradually and ultimately going extinct (Jackson and Sax, 2010). A strict fire regime needs to be initiated and implemented (see Chapter 6 for a proposed Adaptive Management Plan).

7.1.3 Canopy size

The average canopy size of *E. clivicola* plants in Percy Fyfe Nature Reserve is less than half of that of the other populations. This is because of the inability of the reserve management to adhere to the fire regime proposed by Pfab (1997). This particular population is faced with a complex set of factors that hinders its success (Pfab, 1997), which will be discussed later in this chapter and in Chapter 5.

The two fragmented populations, i.e. Radar Hill and Makgeng below R71 road subpopulations have the highest mean canopy sizes of all populations. These sizes indicate that the plants are exposed to unique conditions that promote size expansion. These include: low density (less intraspecific competition), fire (removing apical dominance, i.e., promoting lateral growth), higher herbivory (removing apical dominance, thus promoting meristem growth).

7.1.4 Recruitment capacity

According to Pfab (1997), *E. clivicola* plants reach juvenile stage at approximately six years from seedling establishment. The unavailability of seedlings and juveniles implies that there was no recruitment for over 6 years at the Radar Hill below the road and Makgeng below the R71 road subpopulations starting from at least 2009 to 2014. This is due to the anthropogenic fragmentation of the populations, which resulted in to the disruption of downhill propagule dispersal

Plants from Percy Fyfe Nature Reserve and Radar Hill below the road populations produced flowers, but failed to transform them into fruits. For flowers to develop into fruits pollination/fertilization needs to occur; a process facilitated by pollinators. One common abiotic factor shared by the two populations is low plant density. It is postulated that due to the kinds of pollinators (Hymenoptera-wasps, Formicidae-spiny ant, and Bombylliidae-bee flies) *E. clivicola* is exposed to, pollination success is limited if the populations (*E. clivicola*) remain small. Territory and colony sizes of ants can change due to fluctuations in resource abundance (Gordon, 1995). The Bombylliidae species have been seen to forage as far as 1.5 km from their nests (Wolf and Moritz, 2008). This might be explained by the inability of the plants at the bottom-slope of all fragmented populations to translate flowers into fruits. Due to the low number of individual plants and the distance between them, pollinators are limited.

Pfab (1997) highlighted the fact that the number of cymes and fruits produced by each reproductive plant formed a logarithmic relationship with plants size. This study partially confirmed this in that plants with an area greater than 2000 cm^2 bear the most flowers (74%), but just over half of the flowers (43%) are transformed into fruits. This might be due to the unavailability of pollinators and inaccessibility to the plants, due to graminoids (Chapter 5). Pfab (1997) also elaborated on the fewer fruits observed at Percy Fyfe Nature Reserve population. Pfab (1997) concluded by saying this phenomenon might be due to the extensive herbivory observed at the population. This research added other dimensions to this phenomenon; absence of pollinators and/or graminoids obstruct pollinators to locate *E. clivicola* plants.

This research has identified that the most critical and effective canopy size for *E. clivicola* to bear flowers and successfully transform a greater proportion thereof into fruits range between 200 to 1500 cm^2.

7.2 Abiotic factors

7.2.1 Fire

Africa is often referred to as a 'fire continent' because of the widespread anthropogenic fires that annually burn the savanna vegetation (Sheuyange *et al.,* 2005). Plants evolve traits to adapt to their immediate environmental conditions. Traits, such as resprouting, serotiny and germination by heat and smoke, are adaptives in fire-prone environments. One imported factor to note is that plants are not adapted to fire *per se*, but to fire regimes (Keeley *et al*., 2011).

The effects of fire on *E. clivicola* are addressed in this section as such tackling the research question in Section 3.b of this Chapter. Pfab (1997) has attested to the ability of *E. clivicola* to resprout after experiencing fire. The extent of resprouting is directly proportional to the sustained damage caused by fire. In this study, it was also our observation that fire facilitated the expansion in canopy size of *E. clivicola* plants.

Fire damages mostly the apical tips of *E. clivicola* plants, as such disabling the apical dominance of the shoots. In this case a disabled apical dominance induces new growth from dormant buds or meristems (growth points) (Bangerth, 1994). The newly-developed growth coupled with the branches that are damaged at the tips results in a more dense growth form and concurrently the plants increase in canopy size.

Fire indirectly induces environmental changes through increased resource availability and reduced interspecific competition (Diadema *et al*., 2007). Pfab (1997), has highlighted the relationship between graminoids and *E. clivicola* as a competitive relationship. Due to the short morphological characteristic of *E. clivicola* plants, it can be said that it is hindered in its struggle for sunlight with apically dominant species like graminoids, shrubs and trees. Apically disadvantaged species (*E. clivicola*) also have difficulty in attracting pollinators (Cianciaruso *et al.,* 2012), such as (Hymenoptera-wasps, Formicidae-spiny ant and Bombylliidae-bee flies) to pollinate *E. clivicola* (Pfab, 1997). Fire, herbivory (Chapter 5) and slashing, weaken graminoids, and are prominent reliable mechanisms of maintaining a balance between *E. clivicola* and graminoid completion.

7.2.2 Abiotic features (group cover)

The effects of bare ground, fixed rock and stone cover are tackled to address Section 3.c of this chapter. Percy Fyfe Nature Reserve and Radar Hill below the road populations possessed the greatest proportion of plants (97%) growing on bare ground cover of > 41%. The remaining sampled populations occurred on habitats of < 40% bare ground cover. If bare ground cover is to be correlated with population size or population demography, it would be concluded that bare ground cover is inversely proportional ($P < 0.01$ and $r = -0.52$) to population size; as bare ground cover increases, population size decreases. At the former two populations, less ground cover is observed at the top slopes of the AOO; from top to bottom along the slope the rocky terrain fades away. This is because of the human activities at the foot of the hills, rock weathering is accelerated and, as such, the rocky index is decreased. This phenomenon implies that *E. clivicola* inhibiting the foot hill will decrease in numbers if the human activities occurring at the foot hill persist, because it was postulated earlier in this chapter that, *E. clivicola* is associated with up to 80% rock cover.

A greater proportion of *E. clivicola* plants inhabit areas with a fixed rock cover ranging between 21 and 80%. Increased bare ground promotes emergence of other plant species such as graminoids, shrubs, and trees by providing suitable habitat, thus, intensifying interspecific competition. The fact that *E. clivicola* occurs on habitats with elevated fixed rock cover means that the species avoids competing with other plant species for limited resources (habitat and sunlight). The geological processes of fixed rock weathering produce stones together with macro-nutrients stored in the rocks. Stone cover is associated with fixed rock cover because stones are derived from rocks (rock weathering). Stone formation reduces rock cover and therefore an inverse relationship is observed. Accelerated or anthropogenic rock weathering poses a threat to the *E. clivicola* habitat and ultimately to the species. Limiting human activities on the foothill of *E. clivicola* habitats will foster the establishment and expansion of *E. clivicola* populations.

7.2.3 Aspect and slope

Distribution of plant species is largely controlled by environmental factors, such as water and nutrient availability, light and temperature that may constrain seed germination, seedling survival, growth, and establishment (Warren *et al*., 2013). *E. clivicola* generally prefers to occupy north, north-west, and north-east facing gentle slopes with 1-11° angels. Southern Africa is positioned to the south of the equator (Munyikwa, 2005), as such equator (north-facing) facing slopes are more xeric (warmer, drier and a more variable microclimate) than south-facing slopes. This is because north-facing slopes receive higher solar radiation than their opposite slopes (mesic slopes) (Nevo, 1995). *Euphorbia clivicola* mostly occurs on aspects receiving a high solar radiation. High radiation habitat selection might be due to the short morphological character of the *E. clivicola*. By occupying north-facing slopes with fewer plants, competition for light is minimized. Plant density and canopy size are significantly affected by slope aspect (Badano *et al*., 2005).

It was observed from all sampled populations of *E. clivicola* that, from the top to the bottom of the slope, the canopy size of this species gradually decreased (Section 3.d, page 45, Chapter 4). Size can be used to determine the age of a selected plant species (Pfab, 1997). Gradual differences in canopy size across a slope gradient could mean that the population is expanding down the slope, and the major seed dispersing vector is most probably surface running water. Slope also affects the density of *E. clivicola* plants, because; as the slope gradually descends (moving from top to bottom along the slope) the distance between individuals also increases. This phenomenon implies that the bottom of the slope where *E. clivicola* occurs is of significant importance and no development or destruction should occur on those sites.

Hanski and Gilpin (1997) defined a meta-population as; scattered populations of a species in a landscape, which are in contact via migration. They are characterised by local population extinction and colonisation of unoccupied sites. The obstruction of secondary dispersal mechanism down the slope obstructs the migration and expansion potential of these subpopulations. The fires that occurred in both Radar Hill above road and Makgeng above R71 road excluded the bottom subpopulations, due to the roads that fragmented the subpopulations. The absence of plants on the gravel road is also due to the fact that the road is continuously travelled, all seedlings are trampled by vehicles and commuters, thus no bridging population. This phenomenon implies that there is no contact between fragmented subpopulations of *E. clivicola*. It is further supported by the inability of the flowers to be pollinated at the bottom fragmented populations. The unavailability of contact implies that there is no gene flow, and thus, inbreeding and genetic bottlenecks are foreseen. It may be said that subpopulations of *E. clivicola* fragmented by roads (Radar Hill and Makgeng) do not conform to meta-population dynamics (Chapter 1 and 2).

CHAPTER 5

THE EFFECTS OF BIOTIC FACTORS ON POPULATION BIOLOGY AND ECOLOGY

1. INTRODUCTION

1.1 Biotic factors

Plants are exposed to varying environmental conditions that generally include a combination of biotic (e.g. herbivory, competition, anthropogenic activities) and abiotic factors (e.g. fire, soil, topography, climate) (Martellini *et al*., 2014). Plants not only have acquired mechanisms to contemporaneously cope with biotic and abiotic stress, but probably also to exploit their simultaneous presence to a possible advantage (Atkinson and Urwin, 2012).

1.2 Competition

Pfab (1997), alluded to the compromised survivorship of *Euphorbia clivicola* due to accumulated grass biomass, and it was suggested that this is due to either directly through competition, or indirectly through the alteration of the micro-environment. Rare plant species are particularly vulnerable due to their small population size, poor competitiveness and/or small geographic range (Walck *et al*., 1998). Competition has been recognised as one of the main factors that structure plant populations, plant communities and determine system dynamics (Bittebiere *et al*., 2012). Eliminating one component of a system may sometimes yield undesirable results as was evident in Wellstein *et al.* (2014). Here it observed that when grazing pressure is reduced, some clonal tall grasses tend to prevail and dominate the grassland community through specific plant traits such as tall canopies, extensive lateral spread, and litter deposition.

1.3 Herbivory

Pfab and Witkowski (1999b) recommended that herbivores be removed from the camp where *E. clivicola* occurred at Percy Fyfe Nature Reserve. This recommendation was due to the observed 94% damage to the above-ground branches of *E. clivicola*, presumably by mountain reedbucks (*Redunca fulvorufula*). The competition between *E. clivicola* and graminoids was also highlighted by the ≥ 50% association of grasses with the species under study. Vadigi and Ward (2014) demonstrated the importance of grazers in that they are beneficial for promoting the establishment of other plant species by removing grass competition. Transformation of landscape via urbanisation or any form of construction displaces most herbivores; as such the interaction between graminoids and other plant species is tilted in favour of the graminoids.

1.4 Anthropogenic activities

Anthropogenic influences on habitat can include loss, degradation, fragmentation, and isolation of habitat, which subsequently can affect dynamics and persistence of populations (Hostetler *et al*., 2009). Fragmentation and isolation of ecosystems disrupt biological processes, contributing to the decline of plant populations and sometimes results in changes to the physical and functional interactions within an ecosystem (Newman *et al*., 2013). Plant-pollinator interaction may be compromised because plants in smaller populations, or less dense patches, generally receive fewer visits from pollinators and thus, the availability of pollen may become limiting for seed set (Lamont *et al.,* 1993).

2 RATIONALE

It would be a futile exercise to evaluate the abiotic factors affecting *E. clivicola* (Chapter 4) and not assess the biotic factors' contributing to the ecology of the species, as they are interconnected. In most cases the effects of biotic factors are triggered by abiotic factors and *vice versa*. Pfab (1997) suggested that *E. clivicola* is involved in interspecific competition with graminoids. The rationale for this chapter was to investigate the effects of biotic factors (herbivory, competition and anthropogenic activities) on the biology (life stages, size, flowering, and fruiting) and ecology of *E. clivicola.* The results emanating from this investigation will aid conservators to monitor and manage relevant biotic factors affecting rare and endangered species under study.

3 RESEARCH QUESTIONS

a) How does *Euphorbia clivicola* interact with graminoids?

b) What are the effects of shading on *Euphorbia clivicola*?

c) What are the impacts of human activities on *Euphorbia clivicola*?

4 SPECIFIC AIM AND OBJECTIVES

4.1 Specific aim

This study evaluated the biotic factors affecting the biology and ecology of *Euphorbia clivicola*.

4.2 Specific objectives

This chapter determined the:

a) Grass cover, forb cover, dead vegetation cover, shrub cover and shading that are associated with *Euphorbia clivicola*.

b) Amount of herbivory on *Euphorbia clivicola* and extent of damage that is caused by herbivores.

c) Effect of human activities and other threats to *Euphorbia clivicola*'s persistence.

5 METHODOLOGY

5.1 Grass, forbs, dead vegetation, and shrub cover

All the covers in an area of 15 cm around (micro-habitat) each sampled plant were estimated in terms of percentage of ground covered by each character outlined in the heading above.

5.2 Shading

Shading conditions were evaluated by describing the amount of shading experienced by each sampled plant. The obscured percentage will be determined by the area of the plant that does not receive sunlight at all.

5.3 Herbivory

The presence or absence of damage on each sampled plant was documented. The types of damage were characterised as; herbivory, senescent, wilted, and incinerated (Chapter 4, section 4.2). Senescent plants are those with dry/dead (but still attached) branches. A percentage of such branches were quantified for each sampled plant. Plants that were fed on are those with selected parts of the branches browsed. These plants were classified as a percentage of damaged branches. Preliminary investigations indicated that the animal responsible for herbivory was a small mammal, because the damages observed were specific (only immature and flowering parts of the branches were damaged). These can only be done by an animal with small teeth and mouth parts (Figure 5.1).

Figure 5.1 A *Euphorbia clivicola* plant that was damaged by herbivores.

5.4 Statistical analyses

Statistical analyses parameters described in Chapter 4 were also used in this chapter.

6 RESULTS

6.1 Biotic features

6.1.1 Grass

Grass cover differed significantly between bottom-slope positioned fragmented subpopulations; Radar Hill below road (M = 4.27, $P < 0.05$), Makgeng below R71 road (M = 3.58, $P < 0.05$) and Percy Fyfe Nature Reserve population (M = 4.07, $P < 0.05$) and other (sub)populations. The rest of the populations of *E. clivicola* and *Euphorbia incertae sedis* in Makgeng had a lower grass cover (Radar Hill above road; M = 2.14, $P < 0.05$, Dikgale subpopulation A; M = 2.25, $P < 0.05$, Dikgale subpopulation B; M = 1.92, $P < 0.05$, and Makgeng above R71 road; M = 2.08, $P < 0.05$) (Table 5.1) compared to the above-mentioned populations.

A Post Hoc test (Tukey HSD) revealed grass cover variations between populations; plants at Percy Fyfe Nature Reserve, Radar Hill below the road, and Makgeng below the R71 road populations were surrounded by a significantly higher percentage of ground covered by grass than the latter (Radar Hill above the road, Dikgale and Makgeng above the R71 road) populations (Figure 5.3).

A 30% average of *E. clivicola* plants at Percy Fyfe Nature Reserve, Radar Hill below the road, and Makgeng below R71 road were observed to be covered by a 61—80% of graminoids (Figure 5.2). Radar Hill above road, Dikgale subpopulation A and B, and Makgeng above R71 road populations had 64% of plants that occurred at sites with a 40—60% grass cover (Figure 5.2).

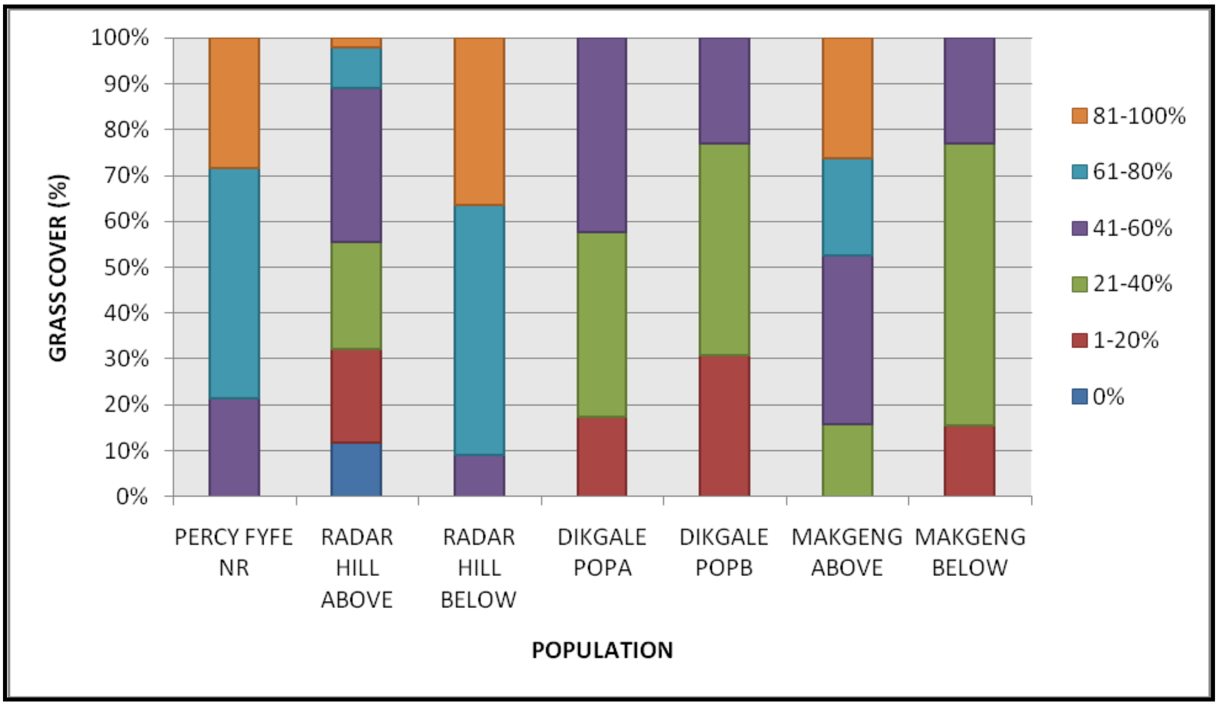


Figure 5.2 The percentage of plants in all known populations of *Euphorbia clivicola* surrounded by different magnitudes of grass cover.

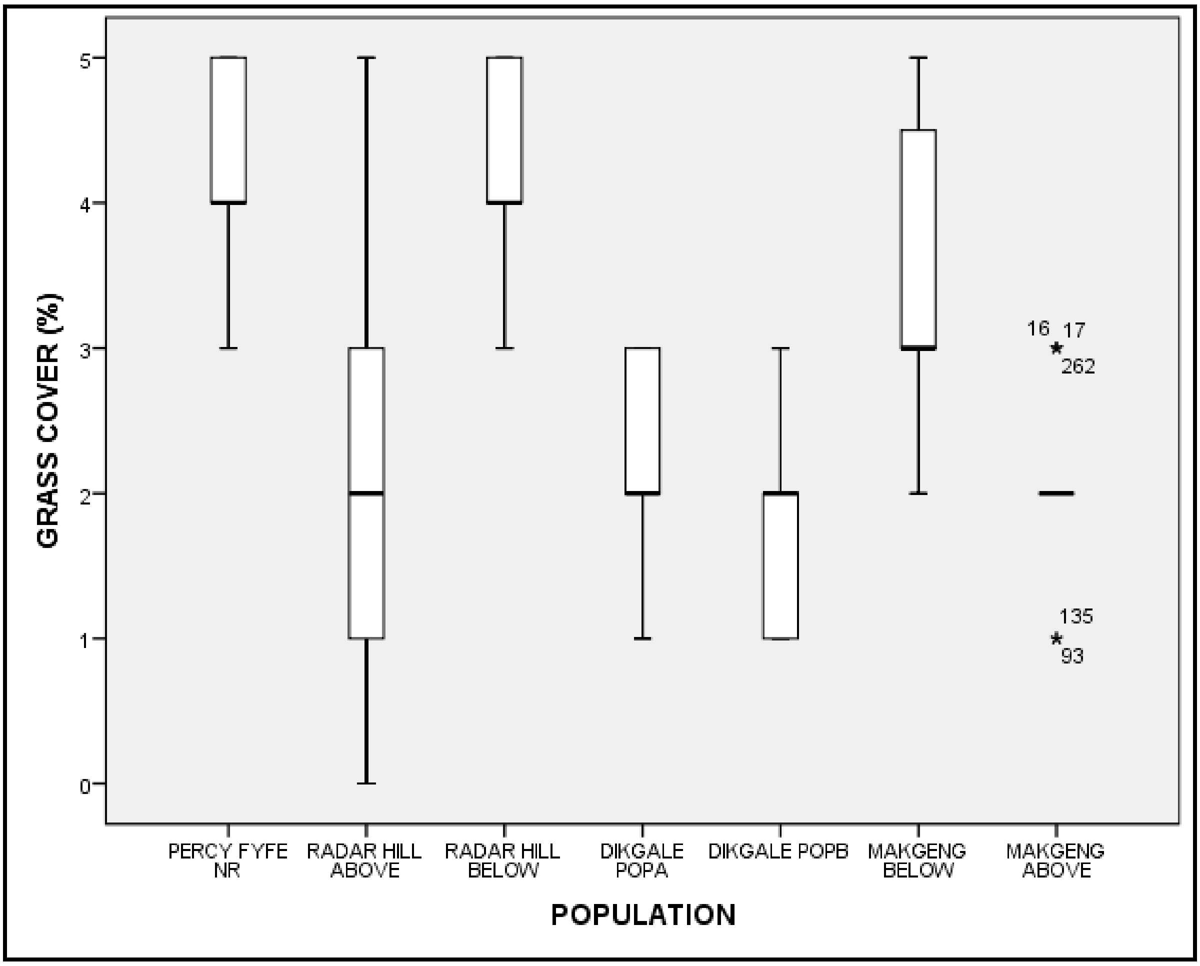


Figure 5.3 Mean grass cover associated with all known populations of *Euphorbia clivicola.*

Life stages of *E. clivicola* and grass cover association were observed in Figure 5.5, and it was evident that adults and senescent plants were associated with a grass cover index of 41—60%(3). Juveniles and seedlings appeared to establish themselves on areas with grass cover of 21—40%(2).

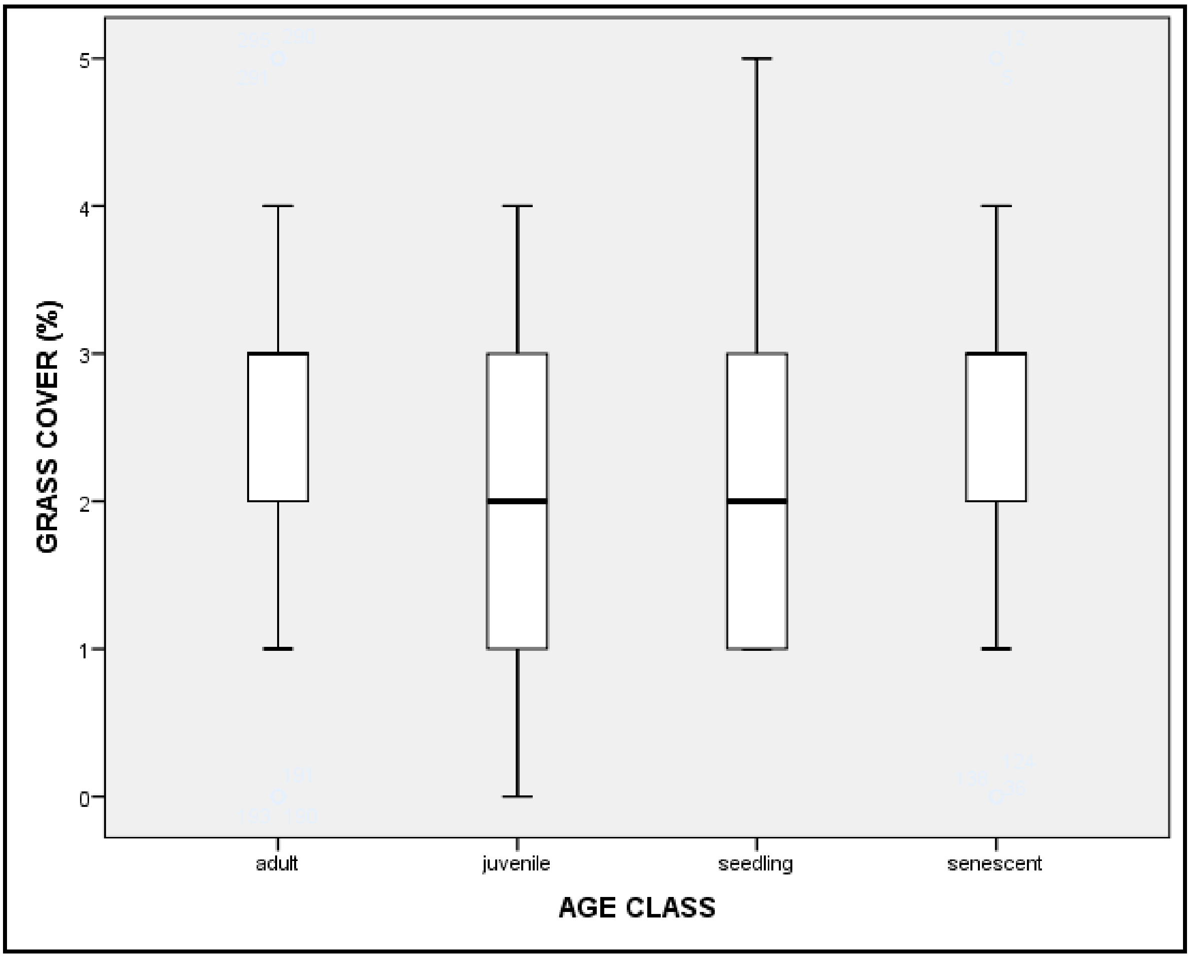


Figure 5.4 Various life stages of *Euphorbia clivicola* and grass cover (20% intervals) around individual plants documented from all known populations.

6.1.2 Forbs

A significant difference was detected between the Radar Hill below road population ($P < 0.05$) and all other populations, except Makgeng below R71 road population. Mean forb cover was observed between Radar Hill below road (M = 2.32) and Makgeng below R71 road (M = 2.73) subpopulations. Radar Hill below road and Makgeng below R71 road subpopulations contained a 51% average of *E. clivicola* surrounded (15 cm radius) by 6—20% of forbs, while Percy Fyfe Nature Reserve had 100% of the plants occurring on micro-habitats of > 5% forb cover (Figure 5.5). The remaining populations had a 79% average of plants inhibiting areas with > 5% forb cover (Figure 5.5).

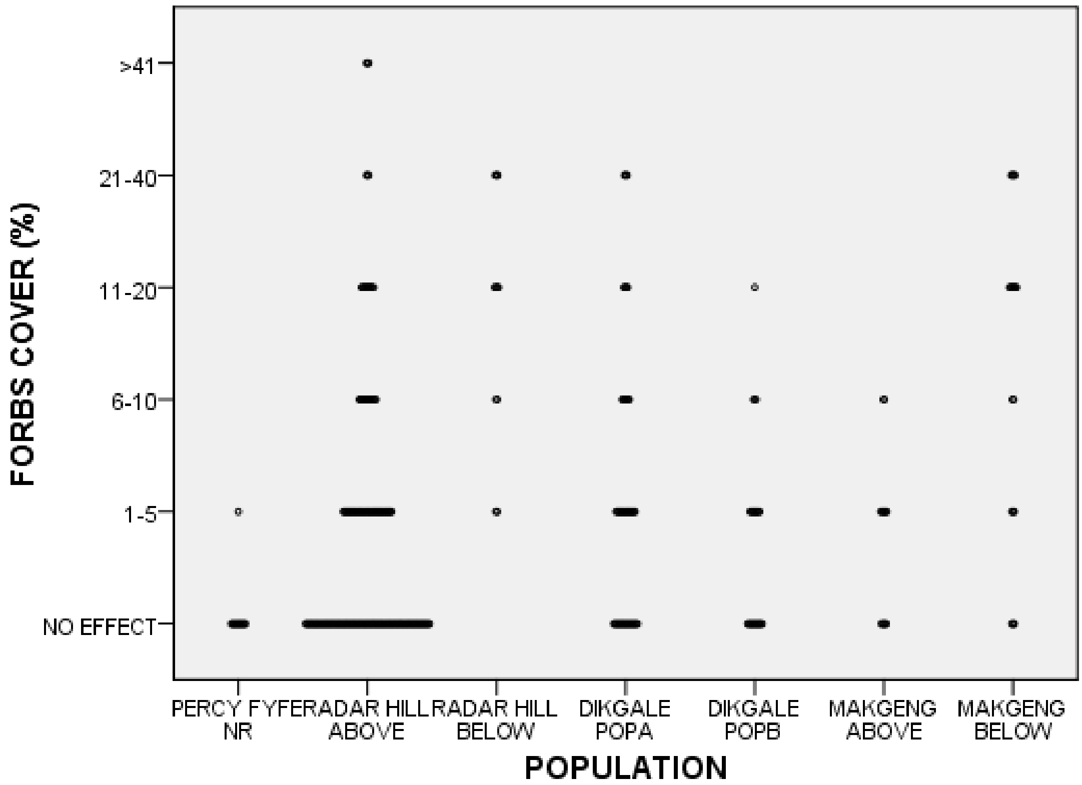


Figure 5.5 The percentage of plants in all known populations of *Euphorbia clivicola* surrounded by different magnitudes of forb cover. The wider width of symbols on the plot represents higher number of sampled plants in that category.

6.1.3 Dead vegetation

More than 66% of *E. clivicola* from Percy Fyfe Nature Reserve, the two Radar Hill, Dikgale and Makgeng below R71 road populations inhabited areas with < 15% dead vegetation (Figure 5.6). Makgeng above R71 road population had 43% of plants occurring in areas with < 15% dead vegetation (Figure 5.6).

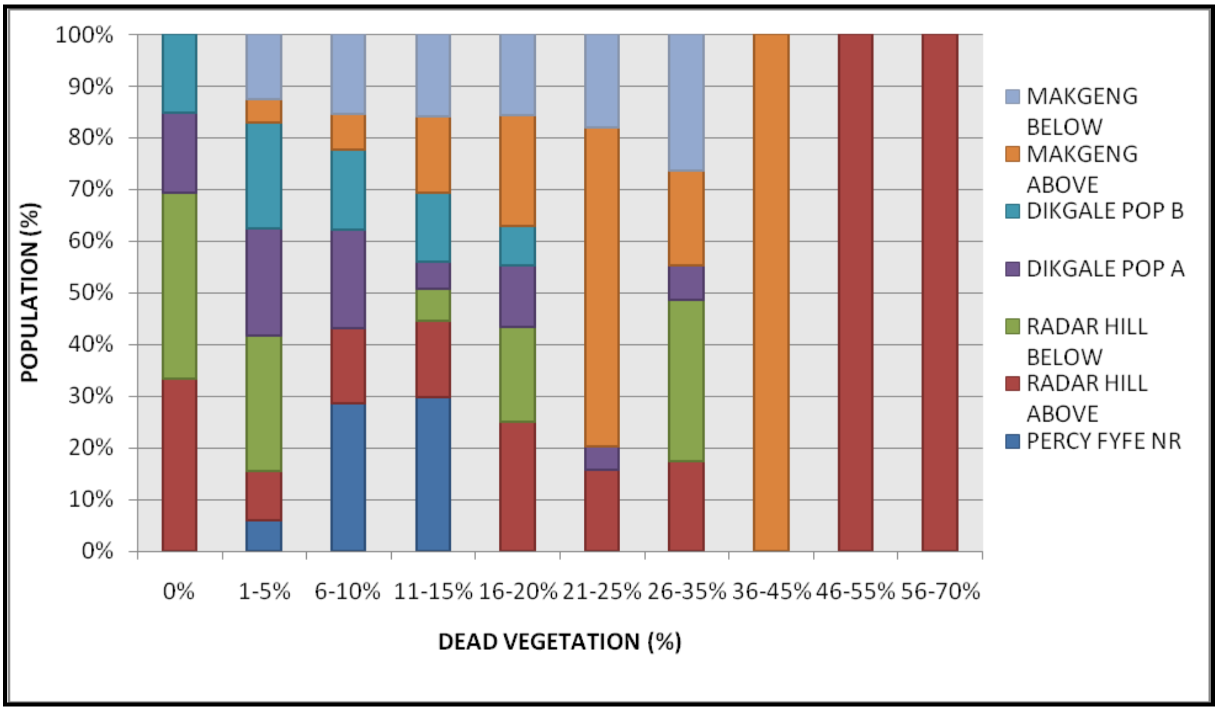


Figure 5.6 The percentage of plants in all known populations of *Euphorbia clivicola* surrounded by different magnitudes of dead vegetation cover.

6.1.4 Shrubs

Percy Fyfe Nature Reserve was the only population without shrubs (Figure 5.7). Dikgale subpopulation A was observed to possess the highest number of shrubs (26%), with a cover of more than 21%. Makgeng above the R71 road possessed (23.1%), Dikgale subpopulation B (19.2%) and Makgeng below the R71 road (10.6%) populations had plants covered by > 21% shrubs. Radar Hill below the road population had 9.1% of plants covered by between 1 and 20% shrubs (Figure 5.7).

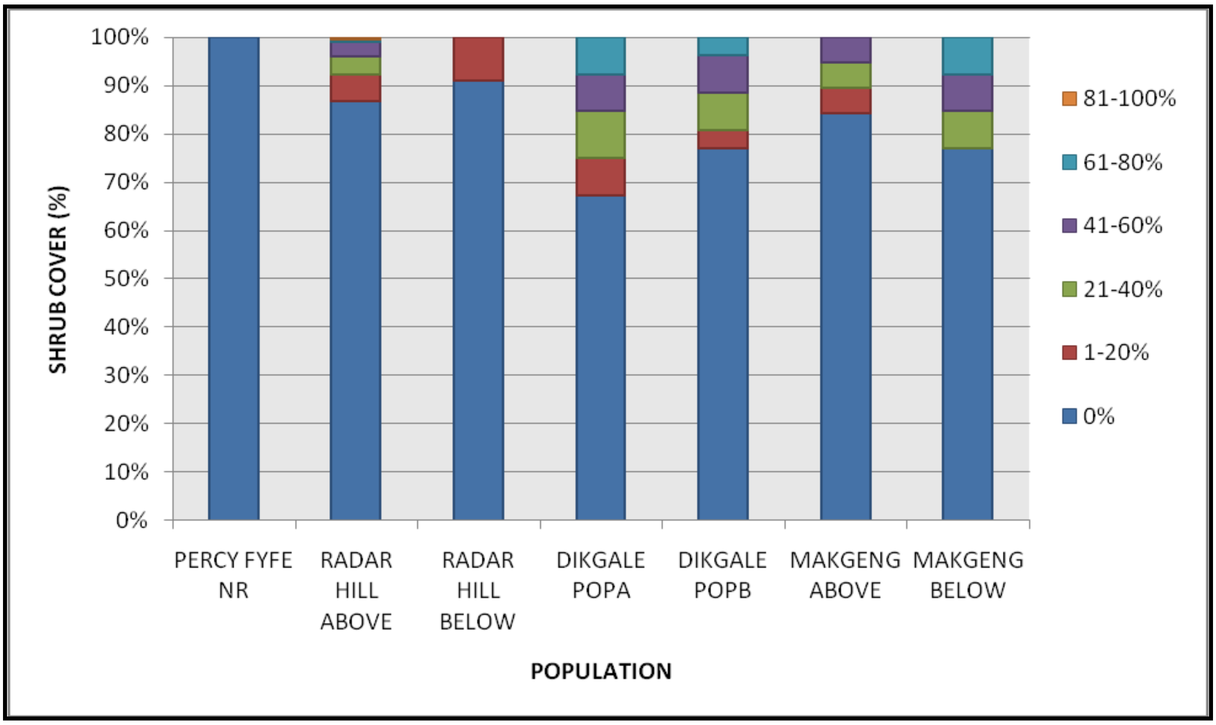


Figure 5.7 The percentage of plants in all known populations of *Euphorbia clivicola* surrounded by different magnitudes of shrub cover.

6.2 Shading

Percy Fyfe Nature Reserve and Radar Hill below road population had an average of 82% plants subjected to > 5% shading, while an average of 75% plants from Radar Hill above road, the two Dikgale, and the two Makgeng populations were subjected to < 5% shading (Figure 5.9). Percy Fyfe Nature Reserve was significantly different ($P < 0.05$) from all other known populations, except the Radar Hill below road population. Percy Nature Reserve (M = 3.29) and Radar Hill population (M = 2.55) were consistent.

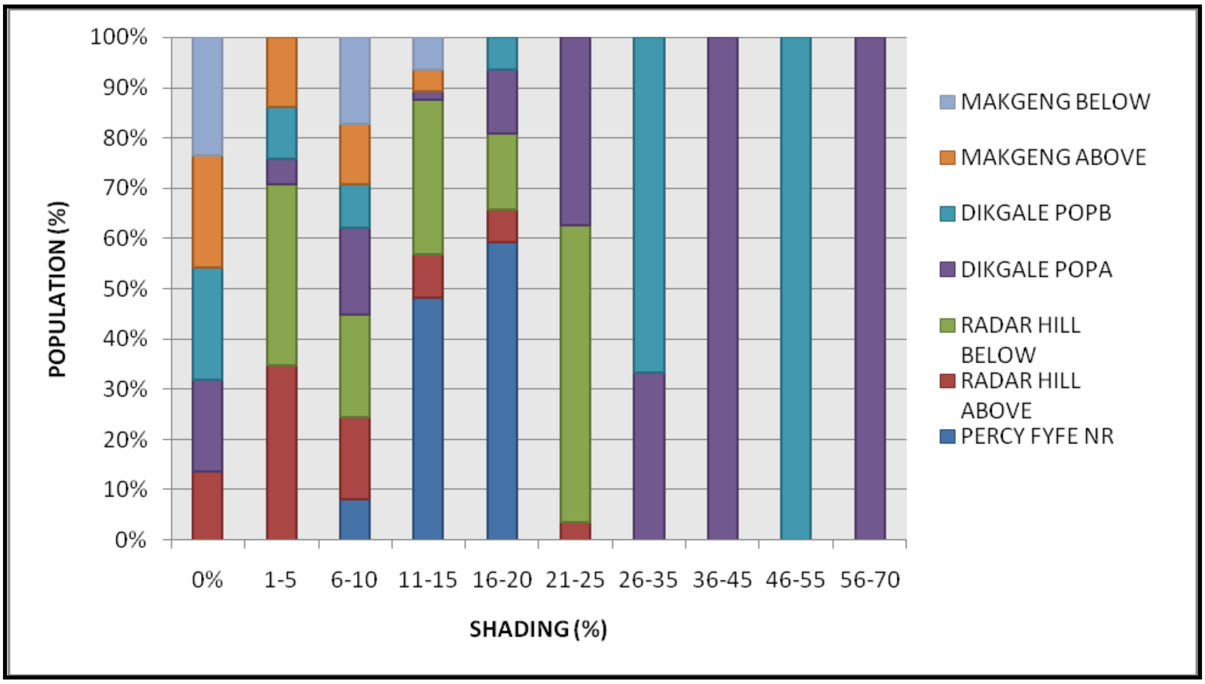


Figure 5.8 The percentage of plants in all known populations of *Euphorbia clivicola* which are obscured from the sunlight.

6.3 Herbivory

Percy Fyfe Nature Reserve, Radar Hill, and Makgeng below R71 road sustained a greater proportion of herbivory (Figure 5.9). Herbivory differed significantly among all known populations of *E. clivicola*, F = 9.98, $P < 0.01$. A Tukey Post Hoc test indicated that herbivory in Percy Fyfe Nature Reserve, Radar Hill above road, the two Dikgale subpopulations, and Makgeng above R71 road differed significantly, $P < 0.05$, while Percy Fyfe Nature Reserve (M = 2.29), Radar Hill below road (M = 2.09), and Makgeng below R71 road (M = 1.47) showed a higher damage from all known populations.

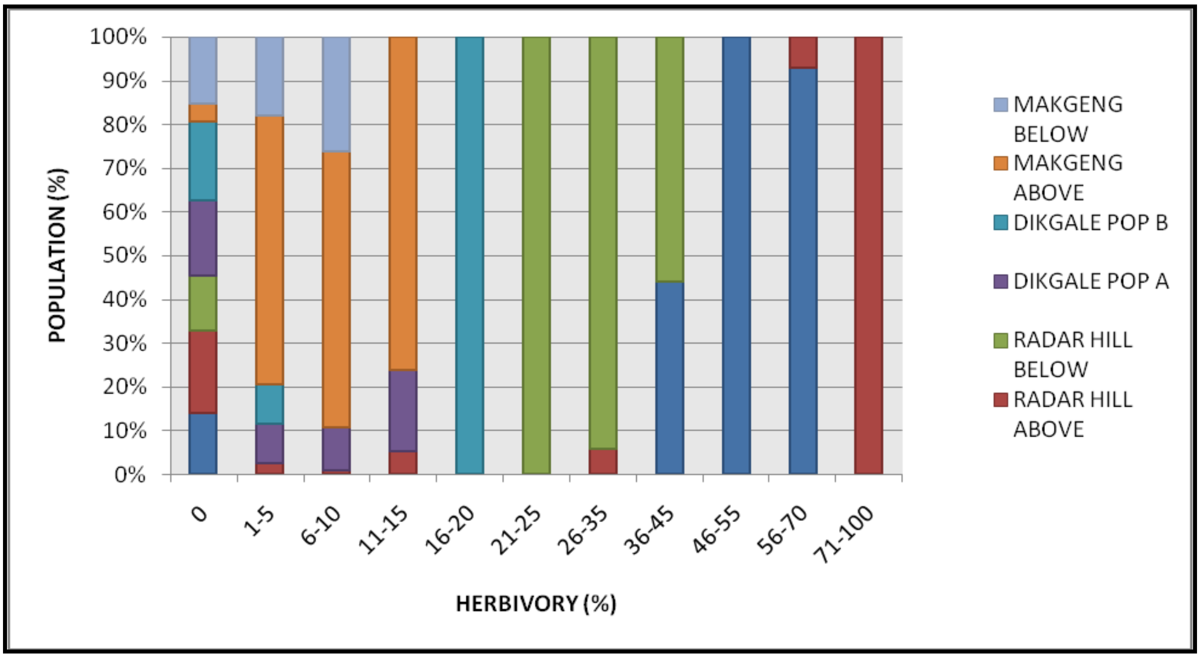


Figure 5.9 Percetage of plants in all known populations of *Euphorbia clivicola,* which sustained various levels of damage by herbivores.

There was a correlation between herbivory and grass cover (Pearson Correlation, $P < 0.01$, $r = 0.19$). It is observed that as the grass cover increases so does the frequency and intensity of herbivory (Figure 5.10).

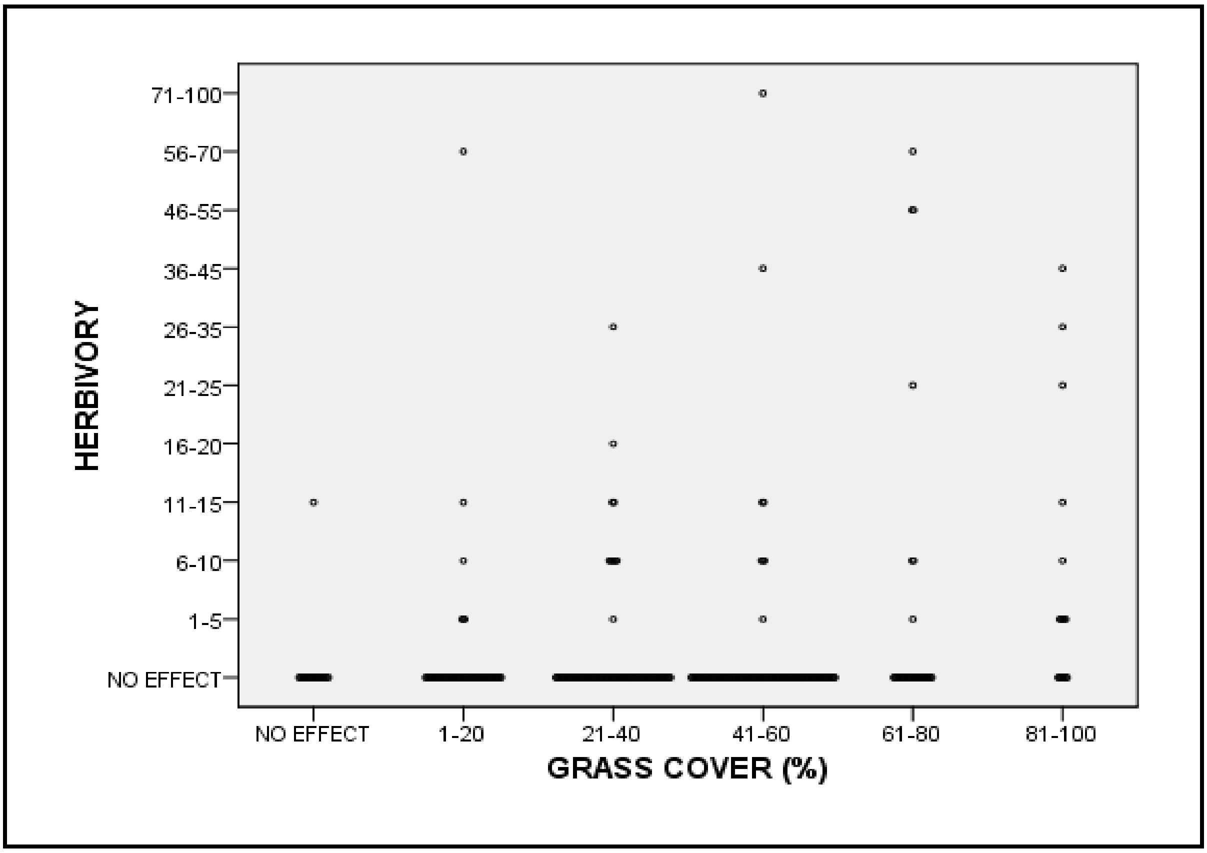


Figure 5.10 The effects of grass cover on herbivory frequency and intensity in all known populations of *Euphorbia clivicola.* The wider width of symbols on the plot represents higher number of sampled plants in that cartegory.

7 DISCUSSION

7.1 Biotic features

7.1.1 Grass

Seedlings of many species require specific regeneration conditions that may differ considerably from those of adult plants (Grubb, 1977). For example, a closed grass sward was identified as one of major obstacles for the successful establishment of less competitive plant species (Donath *et al*., 2006). The interaction between *E. clivicola* and graminoids is elaborated in this section and the research question (Section 3.a) is addressed.

a) Percy Fyfe Nature Reserve population

This population had the second highest grass cover (M = 4.27) after Radar Hill below the road population. The abundance of grasses might be due to the absence of herbivores (see Chapter 5), and also to the inability of the reserve management to adhere to the fire regime as proposed by Pfab (1997). The elevated grass cover has certain detrimental implications for the biology of *E. clivicola*; intraspecific competition, increased density, pollination depression, and refuge for predators (rodents). In addition, Rojas-Sandoval and Meléndez-Ackerman (2012) noted that the high densities attained by grasses and the large amount of litter produced may reduce and even eliminate the occurrence of suitable sites for seed germination and establishment of seedlings.

b1) Radar Hill above the road subpopulation

Grass cover was observed to be M = 2.14 at this population. The moderate grass cover percentage at this population might be as a result of a combination of annual fires and low grazing experienced by the population. Belsky (1986) outlined the benefits of grazers on plants as the following; seed dispersal, soil fertilization, and size reduction of competitive plants. The presence of both seedling and juvenile plants outlined in Chapter 4, Section 5.1.3 indicates that the population is to a certain extent sustainable. Even though, habitat for newly emerging seedlings is limited. The limited habitat for emergence is attributed to the scraped gravel road that fragmented the population in two smaller patches (Figure 2.10).

b2) Radar Hill below the road subpopulation

This population possessed the greatest grass cover (M = 4.27) compared to all known populations of *E. clivicola*. This species is regarded as a rare and critically endangered plant species (Pfab, 1997). It was hypothesised by Moora *et al*. (2003) that rare plants are weaker competitors. It was observed in this study that elevated grass cover triggers the unfolding of the following events: i) flowers are not transformed into fruits (Chapter 4), hence, no seedling and juvenile plants in this subpopulation, and ii) the distance between individual plants is increased due to the grass sward that leaves little to no habitat for seedling emergence.

Over 90% of *E. clivicola* plants occur on aspects with high solar radiation (north-facing). Overgrowth of grasses deprives or out-competes *E. clivicola* of sunlight and as such competition is tilted in the graminoids' favour. This predicament is caused by the anthropogenic fragmentation of the population of Radar Hill that is the scraped gravel road used by mountain bikers. Due to the gravel road, the fire that occurred in August of 2013 (Chapter 4, Section 4.2) did not affect the population below the road, and therefore the number one competitor (graminoids) of *E. clivicola* was never weakened.

c) Dikgale subpopulation A and B

Grass cover at subpopulation A (M = 2.25) was slightly higher than subpopulation B (M = 1.92). Herbivores (cattle) (Figure 5.11) are the only factor controlling the abundance of grasses at these two subpopulations. The slight difference in grass cover might have been due to the fact that construction of a reservoir dam was underway from 2012 to 2014 (Potgieter, unpublished data). Because of that, grazers (cattle) might have tried to avoid confrontation with construction workers, leading to a higher grass cover percentage. It is evident from these subpopulations that herbivores, especially grazers, play a significant role in maintaining the grass cover at a low level, a lesson that Percy Fyfe Nature Reserve Management should take into account (Chapter 6, Section 3.1.3).

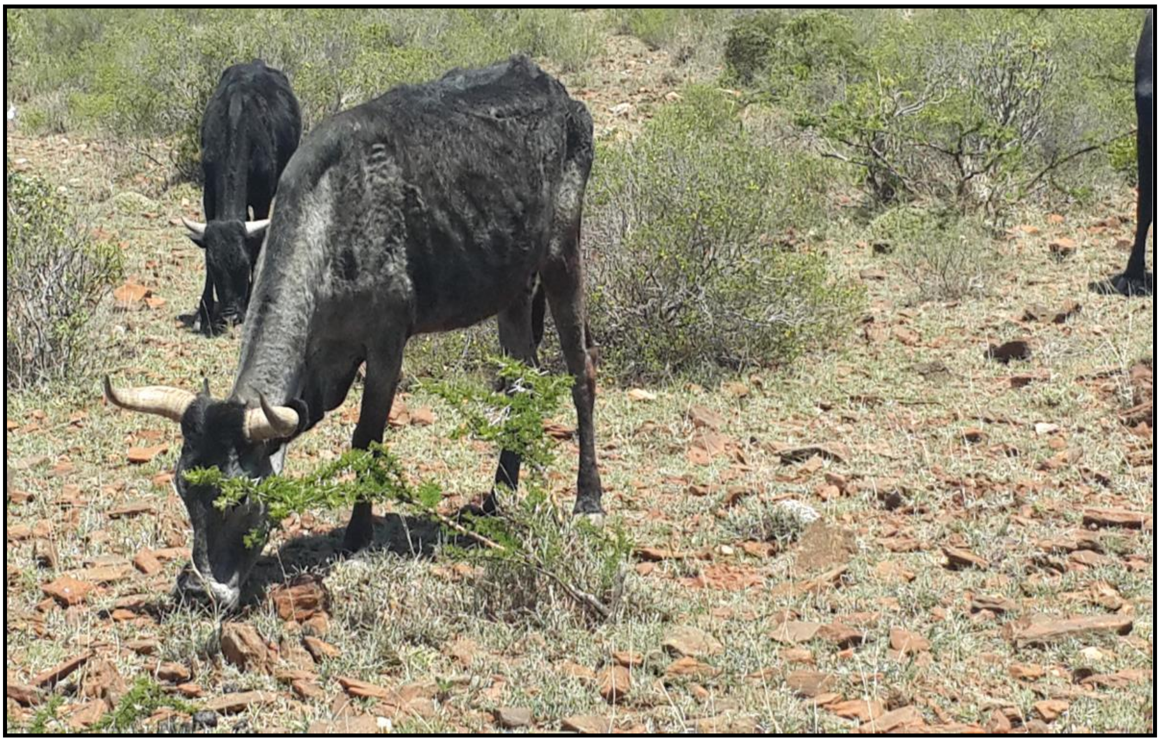


Figure 5.12 Herbivores grazing at Dikgale subpopulation, a locality of *Euphorbia clivicola*.

d1) Makgeng above the R71 road subpopulation

This particular population of *Euphorbia* (*incertae sedis*) was observed to grow on habitat covered by a low grass index (M = 2.08). This low grass cover can possibly be attributed to extensive herbivory (cattle) coupled with annual fires. The fires at this population are to some extent detrimental to the *Euphorbia* species, because they are not timed to coincide with the growing season. This phenomenon is evident because there are more senescent plants than seedlings and juveniles (Chapter 4, Section 5.1.3). When mortality exceeds natality in a population, then the population is said to be unsustainable, and it will gradually decline towards extinction (Waloff and Richards, 1977).

d2) Makgeng below the R71 road subpopulation

The population below the R71 road at Makgeng had the third highest grass cover (M = 3.58) after Percy Fyfe Nature Reserve and Radar Hill below the gravel road. This population faces negative factors similar to those observed in the Percy Fyfe Nature Reserve and Radar Hill below the gravel road populations. A wire fence was erected around the population, making it inaccessible to herbivores. The population was also fragmented by the construction of the R71 road. These two factors contributed to the increased grass cover, which amplified the distance between individual plants, and ultimately depressed regeneration. These features will only cause the population to decline further and ultimately cease to exist (Chapter 6, Section 3.1.3).

Among others, habitat destruction and habitat fragmentation are ongoing major anthropogenic impacts on landscapes, which can strongly affect ecosystems, populations and species (Lienert, 2004). All bottom-slope subpopulations of *E. clivicola* fragmented populations appear to be smothered by grass/graminoids. Habitat fragmentation, leading to small and isolated populations, is considered to be among the most important causes of plant species declines and extinctions (Heinken and Weber, 2013). At Radar Hill a gravel road and at Makgeng a tar road were constructed dividing the *E. clivicola* populations into two subpopulations. The AOO and population demographics are discussed in Chapter 4. Due to the division of the population a fire that occurred in both populations (2013 August) only affected the above road populations and not the below road populations; as such the grass cover in the latter populations was not affected. It is evident from the results that grass cover in Percy Fyfe Nature Reserve, Radar Hill below road, and Makgeng below R71 road were higher.

A high grass cover may make it difficult for pollinators to spot flowering plants and as such reproduction success is minimised. In addition, greater grass cover may prevent germination or outcompete seedlings for resources (sunlight, nutrients and habitat). The absence of seedlings and juveniles at the bottom-fragmented populations may be an indication of the effects of intraspecific competition between graminoids and *E. clivicola* plants. Wherever juveniles and seedlings occurred in *E. clivicola* populations, they were associated with < 5% grass cover.

7.1.2 Forbs, dead vegetation and shrubs

It was also evident that forbs were closely associated with higher grass cover, but Percy Fyfe was an exception. Shrubs were present in all populations of *E. clivicola,* except in Percy Fyfe Nature Reserve, and they did not seem to play any significant role in the population dynamics of *E. clivicola*. The Percy Fyfe Nature Reserve population was particularly low in plant diversity. This inclination implies that establishment of pollinator colonies would be a futile investment, because food sources (flower nectar) are limited in this population. This is also supported by the fact that 100% of the flowers were not pollinated and therefore did not produce fruits in the 2013 field observations.

7.2 Shading

Sunlight provides various beneficial factors to plants. Photosynthesis, triggers flowering in some plant species and facilitates evapotranspiration (Hopkins and Hunter, 2004). Shading is positively related ($P < 0.01$ and $r = 0.26$) to grass cover at Percy Fyfe Nature Reserve and Makgeng below R71 road; as grass cover increased so did shading. Shading obstructed *E. clivicola* from harvesting sunlight (Chapter 5, Section 3.b). As such, an interspecific competition between graminoids and *E. clivicola* for sunlight is suggested. If graminoids out-compete *E. clivicola* then the benefits of sunlight on plants outlined by Hopkins and Hunter (2004) will be diminished for *E. clivicola*, as such compromising the survival of the species.

7.3 Herbivory

The branch sizes of *E. clivicola* are too small to suggest that a big antelope is responsible for harvesting, specifically the tips of branches and/or flower parts. It is thought that small mammals (rodents) are responsible for the damages observed on the tips of *E. clivicola* branches. It was also observed that flowering plants are predominantly browsed and therefore, it is suggested that flowers are more palatable than stems as they don’t possess the milky toxic latex found in stems (Pfab, 1997) and fruits. This is supported by the fact that mountain reedbuck (*Redunca fulvorufula*) are removed from the camp where *E. clivicola* occurs in Percy Fyfe Nature Reserve as Pfab (1997) suggested, but the population still experiences extensive damage caused by herbivores.

Percy Fyfe Nature Reserve, Radar Hill below the road, and Makgeng below the R71 road experienced more extensive herbivory than other populations. Herbivory was found to be positively correlated ($P < 0.01$ and $r = 0.19$) to grass cover, i.e. as grass cover increased so did herbivory frequency and intensity. The increased grass cover is viewed as provided refuge for small herbivores (rodents), hence the positive correlation between the two factors (herbivory and grass cover).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

1 RESEARCH CONCLUSION

From the discussions in all above chapters the following conclusions can be drawn (Figure 6.1):

1.1 Population demographics (Percy Fyfe Nature Reserve and Radar Hill)

This study was motivated by the drastic decline of *Euphorbia clivicola* in the Percy Fyfe Nature Reserve and Radar Hill populations, and the discovery of four new populations two in Dikgale village, Kgwareng and the other two in Makgeng village. Scientific data on the intrinsic and extrinsic factors affecting the biology and ecology of *E. clivicola* were collected from all known populations of the species.

Both of the two formerly known populations of *E. clivicola* (Percy Fyfe Nature Reserve and Radar Hill) have declined drastically in size over the past two decades. The Percy Fyfe Nature Reserve population has declined from an estimated 1921 individuals in 1986 (Raal, 1986) to 165 individuals in 1997 (Pfab, 1997) and recently it possess a bewildering of 8 individuals in 2014. The Radar Hill population declined over 28 years from an initial 3000 individuals in 1986 (Raal, 1986) to an estimate of 382 individuals (Pfab, 1997) with a current population size of 176 individuals. Forty-four percent of *E. clivicola* at the Radar Hill population and 95% of at the Percy Fyfe Nature Reserve have therefore vanished within a period of 18 years (1997—2014). Conservation of these critically endangered species is thus of extreme importance.

1.2 Genetic diversity

Euphorbia clivicola populations from the Percy Fyfe Nature Reserve, Radar Hill, and Dikgale are genetically homogeneous (Chapter 3, Objective 4.2.a, Page 46); and management plans and other conservation resources should therefore be uniform, taking into account the difference in herbivore activity, fire regimes, soil conditions, micro-habitats and surrounding species compositions. These will aid in conservation resources cutback. Transplanting between the above-mentioned populations should be allowed so as to facilitate gene flow and increase the number of individuals at Percy Fyfe Nature Reserve. However, it must be kept in mind that translocation is a complex process and follow up monitoring needs to be done for a period of at least 3—5 years.

Euphorbia schinzii (out-group) is genetically different to other sampled populations (Chapter 3, Objective 4.2.b, Page 46). This clarified the dispute about the identity of *Euphorbia* (*incertae sedis*) plants at Makgeng population. Sampled plants from Makgeng were weakly genetically different from all sampled populations of *E. clivicola*. This diversity might indicate that the Makgeng population is a variety or a sub-species of *E. clivicola*, and a taxonomic study is vital to morphologically characterise the two groups.

1.3 Effects of abiotic factors on population biology and ecology

The combined number of individual plants from the two subpopulations in Dikgale is 60 308, which is the largest and healthiest of all known populations of *Euphorbia clivicola*, followed by Makgeng above the R71 road. The latter population is, however, under threat, because juvenile and seedlings are fewer than senescent plants. The Dikgale subpopulation A is faced with the same fate as the Radar Hill and Makgeng populations because a gravel road was constructed across the xeric (N) facing slope, and therefore, recruitment, migration and expansion of the subpopulation below the road will be constrained, due to the slope descending expansion mechanism of *E. clivicola*.

Canopy size was used to determine the age and success of *E. clivicola* plants. The Percy Fyfe Nature Reserve population has the smallest mean canopy size, which is an indication of the poor performance of the population. The poor performance is due to the domino effect of removing grazers from the camp and failing to implement the three year fire regime. This has resulted in interspecific competition extreme. Radar Hill below the road and Makgeng below the R71 road subpopulations are composed of plants with a significantly large canopy size. This might be due to the inability to recruit because of fragmentation, increased density, or a high grass cover index.

Larger canopy-sized plants produce more flowers but not all of them are pollinated and converted to fruit. This may be due to tall grasses that make it difficult for pollinators to locate the short *E. clivicola*. Increased density is also seen to affect the conversion of flowers into fruits.

The large canopy size is to a certain extent caused by fire, because removal of apical dominance triggers growth from dormant growth points. Fire weakens interspecific competition promoting germination and establishment of seedlings.

Bare ground cover promotes the establishment of graminoids, hence a greater proportion of *E. clivicola* plants prefer habitat with a significantly higher fixed rock and stone cover. This might be to avoid the interspecific competition between *E. clivicola* and graminoids. It was observed that fixed rock cover is inversely proportional to stone cover, and in time rock cover will decrease while stone cover will increase.

A large proportion of *E. clivicola* plants grow on xeric slopes. A xeric slope receives more solar energy than its counterpart (mesic slope). *Euphorbia clivicola* thus prefers a higher solar radiation and drier habitat. Slope was also observed to facilitate migration and possibly seed dispersal (via surface running water). With all that being said, destruction of the north facing slope will negatively affect the success of *E. clivicola*'s persistence.

1.4 Effects of biotic factors on population biology and ecology

The effects of anthropogenic activities on biodiversity can not be overemphasised. Development without proper ecological planning has adversely affected three populations of *Euphorbia clivicola* (Radar Hill, Dikgale, and Makgeng). Development commenced with no specialist studies conducted and environmental management plans were faulty when compiled, because all the above mentioned populations were destroyed on the most sensitive sides (north slopes). The above section addresses the research question in Chapter 5, Section 3c.

Intensive grazing seems to be beneficial for *E. clivicola* at Dikgale population. Grazing eliminates major competitors of the species under study, as does fire. The Percy Fyfe Nature Reserve population was isolated from herbivores and the proposed fire regime is not strictly adhered to. Eliminating one element of an ecosystem triggers other negative or positive effects; in this case an increased grass cover (interspecific completion) which overwhelmed *E. clivicola* at Percy Fyfe Nature Reserve.

Increased grass cover obscures *E. clivicola* plants, reducing available sunlight. This negatively impacts *E. clivicola* which requires high radiation. Graminoids compete with *E. clivicola* for sunlight, habitat and other resources, thus grazing and fire are the only two mechanisms that stabilize the competition between the two species. An increased grass cover also provides refuge for rodents (small mammals) that feed on *E. clivicola*. This is supported by the direct proportionality between herbivory and grass cover. To stop the dire decline and halt the threats on *E. clivicola* populations, management plans need to be developed and implemented.

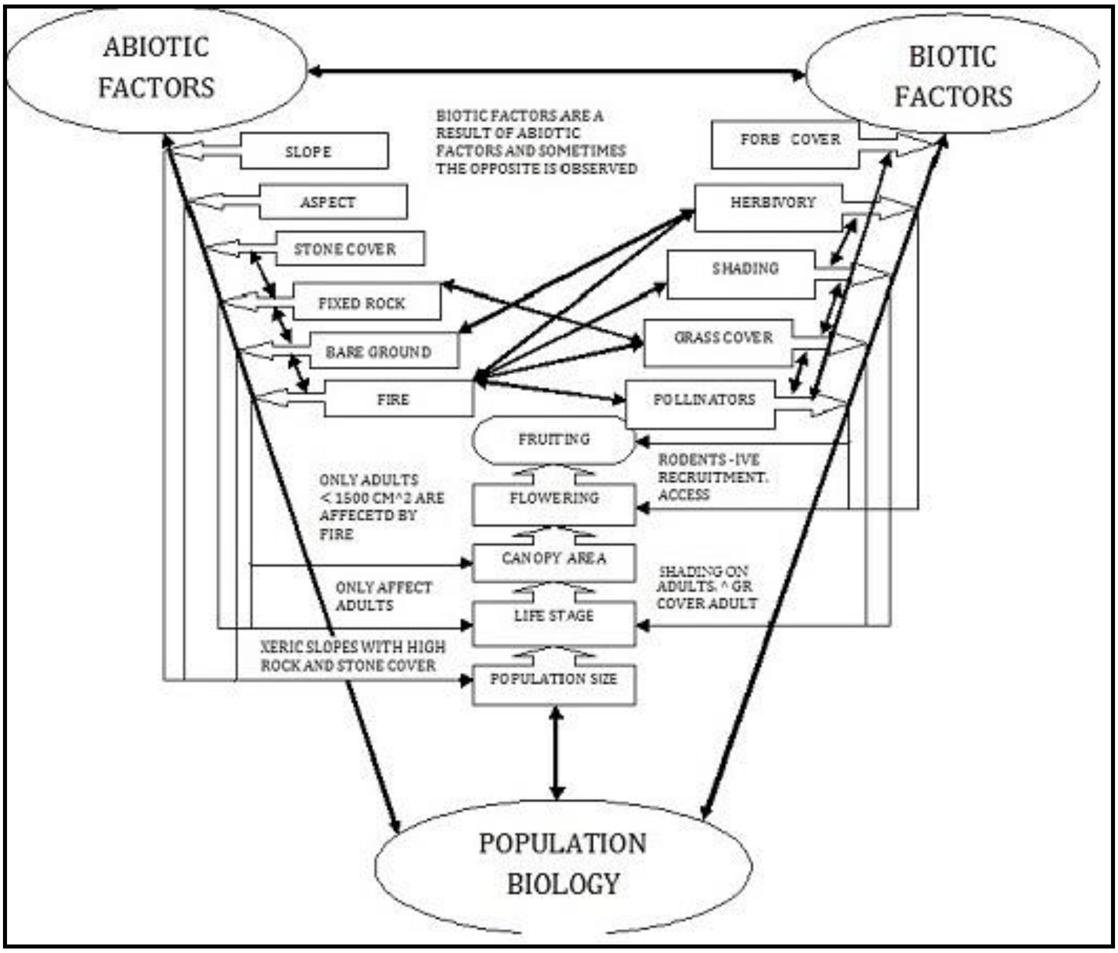


Figure 6.1 A model showing the interactions between abiotic and biotic factors and their implications on the population biology and ecology of *Euphorbia clivicola*.

1.5 Adaptive management

Conservation of threatened plant species relies on four adaptive management tools to effectively conserve such species: a) objectives are developed to describe the desired conditions or outcomes, b) management is designed to meet the objectives, c) the response of the resource is monitored to determine if the objective(s) has/ve been met, and d) management is adapted or (changed) if objectives are met or not (Elzinga *et al*., 1998). The goal of management in any discipline is to control the components of any system under consideration. The components of any system can be controlled only if their characteristics are understood (Harper, 1979). Conservators and management practitioners need to be more aware of research findings, and how they might improve management strategies (Negro *et al*., 2013). Monitoring is inherent in defining adaptive management because management is designed to meet objectives, while monitoring is designed to determine if objectives are met (Elzinga *et al*., 1998).

1.6 Monitoring

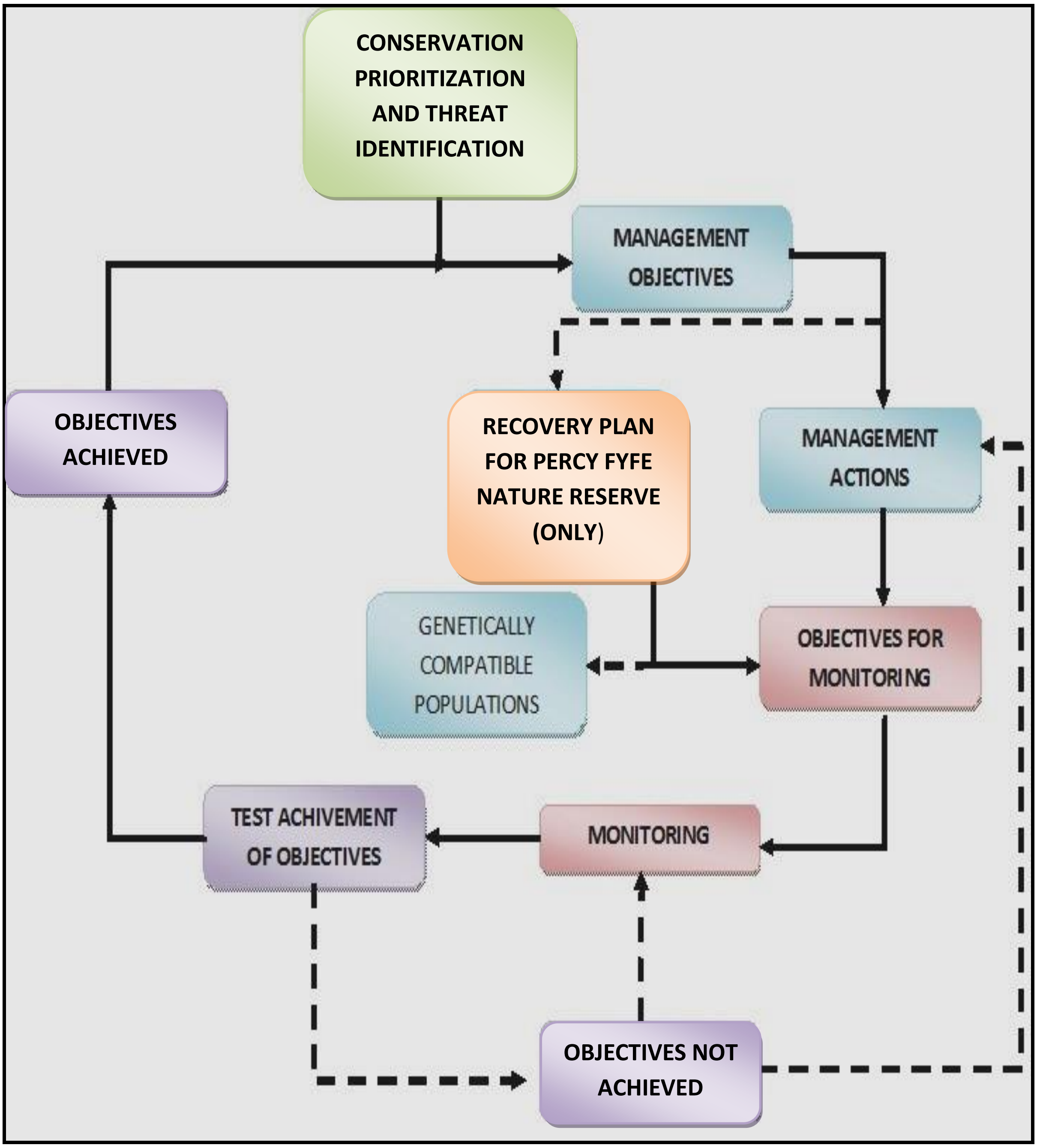
Conservation of rare species requires efforts dedicated to the monitoring of population performance (Lozano *et al*., 2011). Monitoring of biological and ecological factors is divided into three categories: monitoring of each plan's focal species or resource monitoring, monitoring of associated species, and monitoring of habitat (Campbell *et al.*, 2002). Resource monitoring focuses on the plant resource itself and monitors some aspect of that resource, such as population size, average age density, cover, or frequency (Elzinga *et al*., 1998). The associated species category includes monitoring of herbivores, competitors, and exotic species, while habitat monitoring describes how well an activity meets the objectives or management standards for the habitat (Franklin *et al*., 2011).

No known monitoring program of threatened plant species is underway in the Limpopo Province, with cycad spp. being the exception. Elzinga *et al*. (1998) outline three monitoring methodologies as follows: a) Qualitative monitoring which includes estimating quantity and quality, and using a permanent recording method, identifying number of measurement units (macroplots or photoplots), and determining the arrangement of the measurement units (distributed in the population or across the landscape), b) The census monitoring method includes defining the counting units (individuals, stems, clumps, or some other unit) and developing methods to ensure complete counts (transects, plots, or grids), and c) Quantitative studies with sampling entails developing sampling objectives (density, cover, or frequency) with an acceptable level of precision of the estimate (based on the population size). Sampling units must be defined (placed plots or points placed along a line, individual plants, or some other unit), the shape and size of the unit must be described (based on the spatial distribution of the species sampled). A consistent placement method of sampling units must be determined, permanent or temporary sampling units, and estimate the number of sampling units required.

2 RECOMMENDATIONS OF THE STUDY

2.1 Adaptive management plan

The ultimate goal of management in any discipline is control of the components of the system under consideration (Harper, 1979). Species prioritization for conservation is greatly informed by the Red Data List Book, i.e. the species are considered to be threatened by some kind of factor on a certain scale, and active or passive means have to be taken to stabilize or improve their status (Partel *et al.,* 2005). Objectives are developed to describe the desired conditions, while management is designed to meet the objectives, or existing management is continued. The response of the resource is monitored to determine if the objectives have been met. Management is adopted or changed if objectives are not reached (Figure 6.2) (Elzinga *et al.,* 1998).



NB: -------------- Optional/possible pathway

____________ Compulsory pathway

Figure 6.2 Proposed adaptive management plan for *Euphorbia clivicola*.

2.1.1 Conservation prioritization and threat identification

Despite the ambitious goals by IUCN (2007) to significantly reduce the rate of biodiversity loss by 2010, biodiversity continues to be severely threatened (Ramirez-Villegas *et al.,* (2014). Red Data Lists describe how threatened a species is (low number of localities found or low number of individuals), and what the major reasons for its decline may be. Habitat loss, direct exploitation, indirect human influence through changing local ecological interactions, natural disasters, pollution, human population expansion (development) and intrinsic factors have been listed as the main threats (Partel *et al*., 2005). Conservation legislation, however, also include other arguments such as commercial importance and aesthetics.

Euphorbia clivicola is listed in both the Red Data List of South African Plants and the IUCN Red List as critically endangered (Pfab, 1997). This study validates the critically endangered status of this species based on the low number of localities found and the decline in number of individuals. The fact that only one of all known populations of *E. clivicola* is protected and that this protected population (Percy Fyfe Nature Reserve) is mismanaged adds weight to the continued designation of *E. clivicola* as critically endangered. Direct (construction of roads) and indirect (removing herbivores from Percy Fyfe Nature Reserve population) human destruction, as well as interspecific competition (graminoids) are some of the threats posed to *E. clivicola.*

2.1.2 Management objectives

Management objectives are clearly articulated descriptions of a measurable standard, desired state, threshold value, amount of change, or envisaged trend that is strived required for a particular plant population or habitat. Objectives may also set a limit on the extent of an undesirable change and they should be realistic, specific, and measurable (Elzinga *et al.,* 1998).

Elzinga *et al*. (1998), outlined six components required for a complete management objective as: Species or habitat indicator, location, attribute, action, quantity/status, and time frame. A management objective lacking one or more of these components is ambiguous.

In terms of our proposed management plan (Figure 6.2), the following are recommended:

a) Percy Fyfe Nature Reserve population

- Increase the demography (size) of the *Euphorbia clivicola* population at Percy Fyfe Nature Reserve from 8 to 68 individuals though a recovery plan by 2016.
- Promote dispersal and increase the density of the *Euphorbia clivicola* population at Percy Fyfe Nature Reserve to < 2.5 m between individuals between 2015 and 2020.
- Reduce the grass cover from > 60% and maintain it at < 40% at Percy Fyfe Nature Reserve by 2020.

b) Radar Hill population

- Increase the connectivity and habitat size between the two subpopulations of *Euphorbia clivicola* by rehabilitating the road dividing the two subpopulations at Radar Hill between 2016 and 2020.
- Restore the gentle slope state by sealing the ditches at either side of the road at Radar Hill by 2020.

c) Dikgale population

- Increase the habitat size of subpopulation A that was destroyed by a gravel road on the xeric side of the slope between 2015 and 2020.
- Maintain the population sizes of the two subpopulations in Dikgale at the current approximate size of 67 000 individuals of *Euphorbia clivicola* between 2015 and 2020.

- Conscientise the Dikgale tribal authority and Kgwareng community of the conditions and management of their natural resources (*Euphorbia clivicola*) for sustainable use by 2017.

d) Makgeng population

- Increase herbivores' accessibility to the *Euphorbia* (*incertae sedis*) subpopulation below the R71 road to minimize interspecific competition by graminoids, by the beginning of summer 2017.
- Reduce the mortality rate of *Euphorbia* (*incertae sedis*) at Makgeng population above the R71 road by initiating timed fires by 2017.

2.1.3 Management

a) Recovery initiatives for Percy Fyfe Nature Reserve

Recovery is the process that stops the decline of an endangered or threatened species by removing or reducing threats. Recovery ensures the long-term survival of the species in the wild (Bottrill *et al*., 2011).

The demography of *Euphorbia clivicola* at Percy Fyfe Nature Reserve could be increased by re-stocking the population with 60 individual plants of *E. clivicola* (25 from Dikgale subpopulation A, 25 from Dikgale subpopulation B, and 10 from Radar Hill above the road subpopulation) from across the middle slope of selected populations. These subpopulations were selected because of their genetic similarities (Chapter 3) with Percy Fyfe Nature Reserve population and because of their larger demographic sizes.

It is evident from Chapter 4, that *E. clivicola* is dispersed down the slope, and therefore, the transplanted individuals should be planted at the top of a slope. The transplanted individuals should be planted at an average distance of < 2.5 m between them, in so doing, pollination will be encouraged.

Graminoids are believed to be the number one competitor of *E. clivicola* (Chapter 4) (Pfab, 1997). The fire regime alone did not yield positive outcomes at Percy Fyfe Nature Reserve. As such, an alteration of the management plan is proposed; re-introduction of grazers to the camp where *E. clivicola* occurs. Grazing alone has proven to be a beneficial factor for *E. clivicola* at Dikgale population (Chapter 5). Slashing is also an option, but it is a method that has many financial implications. As such, it is not an ideal management strategy for *E. clivicola*. A reduced grass cover will: a) create habitat for emerging seedlings, b) increase access for pollinators, and c) reduce damage (herbivory) by eliminating refuge for rodents.

b) Radar Hill

It was observed from this research project that the roads developed across the populations at Radar Hill and Makgeng inhibited the dispersal of the *E. clivicola* population down the slope. Furthermore, the road excludes the portion of the population below the road from fire, hence, the exponential grass cover observed. It is therefore proposed that for future development or construction to commence where *E. clivicola* and similar species occurs, population migration or expansion direction needs to be determined using the canopy sizes along a gradient and population demographics. No development should begin before such specialist studies are completed.

Ditches were observed on the upper side of the gravel road in Radar Hill, this drainage system redirects the surface water run-off. *E. clivicola* seeds are believed to be dispersed by this surface water run-off, as such, alterations of the slope will disrupt the dispersal of *E. clivicola* propagules. Restoration of the habitat to maintain the gentle slope of the site will aid the dispersal and expansion of the species in question.

c) Dikgale population

This population seemed to be the largest among the *E. clivicola* populations. An eminent threat to the population was observed to be the constructed road at the foot of the hill of subpopulation A. To mitigate this threat, rehabilitation of the road is proposed. This will enhance population expansion and also increase the habitat of the threatened plant. The rehabilitation project will create temporary employment to the inhabitants of Kgwareng village, Dikgale Region.

Conscientising the Tribal authorities and communities where similar rare and threatened species occur will ignite some sense of stewardship towards their surrounding natural resources. The consensus in the wake of the United Nations Conference on Environment and Development (CBD, 2010) suggests that the implementation of what has come to be known as "sustainable development" should be based on local-level solutions derived from community initiatives (Leach *et al*., 1999). An awareness campaign on the biology, ecology and conservation of *E. clivicola* at Dikgale Region is proposed.

d) Makgeng population

Herbivores, especially grazers within *Euphorbia clivicola* habitats have proven to be beneficial in weakening the major competitor (graminoids) *of E. clivicola* (Dikgale population). Removing the fence surrounding the population below the R71 road at Makgeng will give access to herbivores. This will create sufficient habitat for emergence of new seedlings.

As discussed in Chapter 4, plant species do not really adapt to fire but to a fire regime. Untimed and uncontrolled fires affect the *E. clivicola* at Makgeng population above the R71 road negatively. Mortality supersedes natality, which means the population is not sustainable. A controlled fire regime of burning once every year after the last cycle of fruiting on the first week of October is proposed.

2.1.4 Monitoring objectives

a) Percy Fyfe Nature Reserve population

- A distance of < 2.5 m between individuals of *Euphorbia clivicola* at Percy Fyfe Nature Reserve is attained between 2015 and 2020.

- The grass cover at Percy Fyfe Nature Reserve will be reduced from > 60% to < 40% and this should be attained between 2015 and 2020.

b) Radar Hill population

- A 70% assurance that the road and ditches at Radar Hill are restored should be accomplished by 2020.

c) Dikgale population

- A 70% assertion that the road at Dikgale subpopulation A is rehabilitated should be realised by 2020.
- A confidence of 60% that the population size at Dikgale is maintained at 67 000 individuals between 2015 and 2020.
- There should be a 95% certainty that the community of Kgwareng is aware of the status and management of *Euphorbia clivicola* by 2016.

d) Makgeng population

- A 95% confidence that the intensity of grazing is increased should be attained between 2015 and 2020.
- A confidence of 80% that seedlings and juveniles are greater than senescent at the population above the R71 road in Makgeng.

2.1.5 Monitoring

Conservation of rare species requires efforts dedicated to monitoring of population performance (Lozano *et al*., 2011). Monitoring of *E. clivicola* is proposed to focus on three monitoring objectives: a) Density; the number and distance between *E. clivicola* plants in sample units should be counted in order to detect vital rates of population growth. The information from this sample of plants will be used to approximate the general trend in the size of the population (increasing, decreasing, or stable) (Chapter 4, methodology) b) Demographic structure; the number of *E clivicola* in each of the four life stages (seedling, juvenile, adult, and senescent) will be counted in order to better understand changes in the demographic structure (e.g. a decline in recruitment or a decline in all life stages). Demographic structure monitoring can often detect population changes earlier than simple density monitoring (Elzinga *et al*., 1998), c) Inter-specific competition; the plant species that co-occur (graminoids) with *E. clivicola* as well as their cover in the sampling units will be recorded in order to characterize *E. clivicola* habitat and associated plant communities. Changes in the percentage cover of co-occurring species and the total vegetative cover within the monitoring units will identify the direction of inter-specific competition pressure on *E. clivicola* populations.

a) Percy Fyfe Nature Reserve

A count of *E. clivicola* plants should be conducted annually during May and June to coincide with flowering and fruiting seasons. Transects that run from bottom to top slope should be placed 10 m apart, and the following parameters should be measured: a) Age class, b) Distance between individual plants (density), c) Count individual flowers and fruits on each sampled plant, d) Canopy size and, e) Grass cover in a 15 cm radius around individual plants (Chapter 4 and 5).

b) Radar Hill

The ultimate goal of rehabilitating the road and ditches at this population is to connect the two subpopulations of *E. clivicola*, as such, increasing the habitat. A successful rehabilitation program will be achieved when the top subpopulation is able to disperse or expand down slope. A total species count should be conducted annually between April and June measuring the same parameters as at the Percy Fyfe Nature Reserve. The dispersal across the road will be indicated by the emergence of seedlings at the below road population fragment.

c) Dikgale

The road should be rehabilitated to meet the standards outlined for Radar Hill. Based on the enormous size of the two subpopulations, the subpopulations will be sampled using a Point Centre Quadrat Method (Chapter 4). Fifty percent of the subpopulations should be sampled annually between April and July. The same parameters as at the Percy Fyfe Nature Reserve and Radar Hill should be measured. A workshop on the Community Based Resource Management (*E. clivicola* management) should be initiated between 2015 and 2017. A questionnaire assessment should be conducted after the workshop to consider if any amendments should be made to the management of *E. clivicola*.

d) Makgeng

To assess whether or not grazing pressure is increased at the subpopulation below the R71 road; grass cover in a radius of 15 cm surrounding individual plants should be measured annually after the removal of the wire fence around the subpopulation. An annual demographic assessment (as Percy Fyfe Nature Reserve) is proposed between January and March, during the emergence of seedlings.

Upon the completion of the first monitoring data set, the data should be analysed and tested to determine whether or not it achieved the management objectives. If it achieved them, then the management and monitoring is adopted, but if the management objectives were not realised then alternative management actions should be formulated.

3 AREAS OF FUTURE RESEARCH

- More ISSR primers must be used to determine DNA diversity intra-populations and a different *Euphorbia* spp be used as an outgroup.
- Taxonomic and DNA classification of *Euphorbia* spp. (*incertae sedis*) at Makgeng populations.
- Land restoration (rehabilitation) of degraded habitats of *Euphorbia clivicola*.
- Re-stocking the Percy Fyfe Nature Reserve population of *Euphorbia clivicola* by Relocation (transplanting) plants from genetically compatible populations.
- Fine-scale distribution patterns of *Euphorbia clivicola*.

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